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Date: *11/24/97*   By: _____
                               Karen Bellinger

Assistant Commissioner for Patents
Washington, D.C. 20231

Box Patent Application

Sir:

1.   Transmitted herewith for filing is a:

    a.   [X]   utility application

        []   design patent application

        []   plant patent application

    b.   Inventor(s): Nelson B. Freimer; Lodewijk A. Sandkujil; Pedro Leon; Victor I. Reus; Michael Escamilla; Lynne Allison McInnes; Susan K. Service

    c.   For: METHODS FOR TREATING BIPOLAR MOOD DISORDER ASSOCIATED WITH MARKERS ON CHROMOSOME 18P

2.   Enclosed are:

    a.   [X]   <u>45</u> sheets of [X] informal [] formal drawing(s).

    b.   [X] an unexecuted Declaration(s) and Power(s) of Attorney.

    c.   []   A Verified Statement to establish small entity status under 37 CFR 1.9 and 37 CFR 1.27. [] Along with a copy of the assignment, which is <u>not</u> to be recorded.

    d.   []   Certified copy(ies) of application number(s) _ filed .

    e.   []   An Information Disclosure Statement.

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1.

f. [] A Preliminary Amendment.

g. [] A Petition for Expedited Foreign Filing License.

h. [] A Declaration of Availability under MPEP 608.01(p)C.

i. [] An Associate Power of Attorney.

j. [] A duplicate copy of this plant patent application.

k. [] .

3. This application is a:

[] divisional application

[X] continuation application

[] continuation-in-part application

of U.S. Application Serial No. 08/916,683, and [] a Petition for Extension of Time for filing in the earlier application is enclosed.

Please amend the specification by inserting on page 1, line 3, the following cross-reference to related applications:

-- <u>CROSS-REFERENCE TO RELATED APPLICATIONS</u>

This application is a continuation application of U.S. Application Ser. No. 08/916,683, filed August 22, 1997 and claims the benefit of the filing date of United States Provisional Application Ser. No. 60/023,438, filed August 23, 1996.--

4. The filing fee has been calculated as shown below:

[] The filing fee for a design application:

	Small Entity	Other than a Small Entity
[]	$165.00	
[]		$330.00

Design Application Fee: $

[] The filing fee for a plant patent application:

	Small Entity	Other than a Small Entity
[]	$270.00	
[]		$540.00

Plant Patent Application Fee: $

[X] Utility application:

CLAIMS AFTER ENTRY OF ANY AMENDMENTS, LESS ANY CANCELLED CLAIMS

FOR:	Claims Filed		Extra Claims[1]	Small Entity Rate	Fee	Other Than a Small Entity Rate	Fee	Total Filing Fee
Basic Fee					$395		$790	$790.00
Total Claims	16	-20=	0	$11		$22		$0.00
Independent Claims	7	-3=	4	$41		$82		$328.00
Multiple Dependent Claims Presented				$135		$270		$0.00
TOTAL								$1,118.00

1 If difference is negative, enter "0".

[] Petition for Expedited Foreign Filing License ($130.00) $

Other fees (list individually)

   (none)

Total Other Fees: $

TOTAL FEES: $1,118.00

[X]   A check for the amount of the above indicated TOTAL FEES is attached.

[]   Please charge Deposit Account No. 03-3117 in the amount of $.

[]   A check in the amount of $ is attached.

[]   No fee is required.

[X]   <u>Conditional Petition for Extension of Time</u>: An extension of time is requested to provide for timely filing <u>if</u> an extension of time is still required after all papers filed with this transmittal have been considered.

[X]   The Commissioner is hereby authorized to charge any underpayment of the following fees associated with this communication, including any necessary fees for extension of time, or credit any overpayment to Deposit Account No. 03-3117:

      [X]   Any filing fees under 37 CFR 1.16 including fees for the presentation of extra claims.

      [X]   Any patent application processing fees under 37 CFR 1.17.

A **duplicate** copy of this sheet is attached for accounting purposes.

Respectfully submitted,

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1 METHODS FOR TREATING BIPOLAR MOOD DISORDER

2 ASSOCIATED WITH MARKERS ON CHROMOSOME 18p

3

4 <u>ACKNOWLEDGEMENTS</u>

8

9 <u>INTRODUCTION</u>

10

11 <u>Background</u>

12

13 **BIPOLAR MOOD DISORDER (BP)**

14 Manic-depressive illness, or bipolar mood disorder (BP), is characterized by episodes

15 of elevated mood (mania) and depression and is among the most prevalent and potentially

16 devastating of psychiatric syndromes. The most severe and clinically distinctive forms of BP

17 are BP-I (severe bipolar mood disorder) and SAD-M (schizoaffective disorder manic type),

18 and are characterized by at least one full episode of mania, with or without episodes of major

19 depression (defined by lowered mood, or depression, with associated disturbances in

20 rhythmic behaviors such as sleeping, eating, and sexual activity). A milder form of BP is

21 BP-II, bipolar mood disorder with hypomania and major depression. BP-I often co-

22 segregates in families with more etiologically heterogeneous syndromes, such as unipolar

23 major depressive disorder (MDD), which is a more broadly defined phenotype. See

24 McInnes, L.A. and Freimer, N.B., Mapping genes for psychiatric disorders and behavioral

25 traits, Curr. Opin. in Genet. and Develop., 5:376-381 (1995).

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1.

TREATMENT OF INDIVIDUALS WITH BIPOLAR MOOD DISORDER

An estimated 2-3 million people in the United States are affected by BP-I. Currently, individuals are typically evaluated for bipolar mood disorder using the clinical criteria set forth in the most current version of the American Psychiatric Association's <u>Diagnostic and</u> <u>Statistical Manual of Mental Disorders</u> (DSM). Many drugs have been used to treat individuals diagnosed with bipolar mood disorder, including lithium salts, carbamazepine and valproic acid. However, none of the currently available drugs is able to treat every individual diagnosed with severe BP-I (termed BP-I) and drug treatments are effective in only approximately 60-70% of individuals diagnosed with BP-I. Moreover, it is currently impossible to predict which drug treatments will be effective in particular BP-I affected individuals. Commonly, upon diagnosis affected individuals are prescribed one drug after another until one is found to be effective. Early prescription of an effective drug treatment is critical for several reasons, including the avoidance of extremely dangerous manic episodes and the risk of progressive deterioration if effective treatments are not found. Also, appropriate treatment may prevent depressive episodes in BP-I individuals; these episodes are also dangerous and are characterized by a high suicide rate. The high prevalence of the disorder, together with frequent occurrence of hospitalizations, psychosocial impairment, suicide and substance abuse, has made BP-I a major public health concern.

Genetic Basis for Bipolar Mood Disorder

Mapping genes for common diseases believed to be caused by multiple genes, such as BP-I, may be complicated by the typically imprecise definition of phenotypes, by etiologic heterogeneity and by uncertainty about the mode of genetic transmission of the disease trait. With psychiatric disorders there is even greater ambiguity in distinguishing between individuals who likely carry an affected genotype from those who are genetically unaffected. For example, one can define an affected phenotype for BP by including one or more of the broad grouping of diagnostic classifications that constitute the mood disorders: BP-I, SAD-M, MDD, and BP-II.

Thus, one of the greatest difficulties facing psychiatric geneticists is uncertainty regarding the validity of phenotype designations, since clinical diagnoses are based solely on

1   clinical observation and subjective reports. Also, with complex traits such as psychiatric

2   disorders, it is difficult to map the trait-causing genes genetically because: (1) the BP-I

3   phenotype doesn't exhibit classic Mendelian recessive or dominant inheritance patterns

4   attributable to a single genetic locus, (2) there may be incomplete penetrance i.e., individuals

5   who inherit a predisposing allele may not manifest the disease; (3) the phenocopy

6   phenomenon may occur, i.e., individuals who do not inherit a predisposing allele may

7   nevertheless develop the disease due to environmental or random causes; (4) genetic

8   heterogeneity may exist, in which case mutations in any one of several genes may result in

9   identical phenotypes.

10   The existence of one or more major genes associated with BP-I and with a clinically

11 similar diagnostic category, SAD-M (schizoaffective disorder manic subtype), is supported by

12 segregation analyses and twin studies (Bertelson et al., 1977; Freimer and Reus, 1992; Pauls

13 et al., 1992). However, efforts to identify the chromosomal location of BP-I genes have

14 yielded disappointing results in that reports of linkage between BP-I and markers on

15 chromosomes X and 11 could not be independently replicated nor confirmed in the re-

16 analyses of the original pedigrees (Baron et al., 1987; Egeland et al., 1987; Kelsoe et al.,

17 1989; Baron et al., 1993). The possible localization of BP genes on chromosomes 18

18 (pericentromeric region) and 21q has been suggested, but in both cases the proposed

19 candidate region is not well defined and there is equivocal support for either location

20 (Berrettini et al. (1994) Proc. Natl. Acad. Sci. USA, 91, 5918-5921, Murray, J.C., et al.

21 (1994) Science 265, 2049-2054; Pauls et al., Am. J. Hum. Genet. 57:636-643 (1995); Maier

22 et al., Psych. Res. 59:7-15 (1995); Straub et al., Nature Genet., 8:291-296 (1994)). Recent

23 investigations have led to the isolation of chromosome 18-specific brain transcripts which

24 have been suggested to be positional candidates for bipolar disorder (Yoshikawa et al., Am.

25 J. Med. Gen. 74, 140-149 (1997)).

26   Despite abundant evidence that BP has a major genetic component, linkage studies

27 have not yet succeeded in definitively localizing a BP gene. This is mainly because mapping

28 studies of psychiatric disorders have generally been conducted under a paradigm appropriate

29 for mapping genes for simple Mendelian disorders, namely, using linkage analysis in the

30 expectation of finding high lod scores that definitively signpost the location of disease genes.

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1   The follow up to early BP linkage studies, however, showed that even extremely high lod

2   scores at a single location can be false positives. See Egeland, et al., Nature 325:783-787

3   (1987); Baron et al., Nature 326:289-292 (1987); Kelsoe et al., Nature, 342:238-243 (1989);

4   and Baron et al., Nature Genet. 3:49-55 (1993). These earlier studies used largely

5   uninformative markers and did not use stringent criteria for identifying affected individuals.

6

7   LINKAGE DISEQUILIBRIUM ANALYSIS

8   Linkage disequilibrium (LD) analysis is a powerful tool for mapping disease genes

9   and may be particularly useful for investigating complex traits. LD mapping is based on the

10   following expectations: for any two members of a population, it is expected that

11   recombination events occurring over several generations will have shuffled their genomes, so

12   that they share little in common with their ancestors. However, if these individuals are

13   affected with a disease inherited from a common ancestor, the gene responsible for the

14   disease and the markers that immediately surround it will likely be inherited without change,

15   or IBD ("identical by descent"), from that ancestor. The size of the regions that remain

16   shared (i.e. IBD) are inversely proportional to the number of generations separating the

17   affected individuals and their common ancestor. Thus, "old" populations are suitable for fine

18   scale mapping and recently founded ones are appropriate for using LD to roughly localize

19   disease genes more approximately (Houwen et al., 1994, in particular Fig. 3 and

20   accompanying text). Because isolated populations typically have had a small number of

21   founders, they are particularly suitable for LD approaches, as indicated by several successful

22   LD studies conducted in Finland (de la Chapelle, 1993).

23   LD analysis has been used in several positional cloning efforts (Kerem et al., 1989;

24   MacDonald et al., 1992; Petrukhin et al., 1993; Hastbacka et al., 1992 and 1994), but in

25   each case the initial localization had been achieved using conventional linkage methods.

26   Positional cloning is the isolation of a gene solely on the basis of its chromosomal location,

27   without regard to its biochemical function. Lander and Botstein (1986) proposed that LD

28   mapping could be used to screen the human genome for disease loci, without conventional

29   linkage analyses. This approach was not practical until a set of mapped markers covering

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1 the genome became available (Weissenbach et al., 1992). The feasibility of genome

2 screening using LD mapping is now demonstrated by the applicants.

3 Identification of the chromosomal location of a gene responsible for causing severe

4 bipolar mood disorder can facilitate diagnosis, treatment and genetic counseling of

5 individuals in affected families.

6 Due to the severity of the disorder and the limitations of a purely phenotypic

7 diagnosis of BP-I, there is a tremendous need to subtype individuals with BP-I genetically to

8 confirm clinical diagnoses and to determine appropriate therapies based on their genotypic

9 subtype.

10

11 ## SUMMARY OF THE INVENTION

12 The present invention comprises using genetic linkage and haplotype analysis to

13 identify an individual having a bipolar mood disorder gene on the short arm of chromosome

14 18. In addition, the present invention provides markers linked to a gene responsible for

15 susceptibility to bipolar mood disorder that will enable researchers to focus future analysis on

16 that small chromosomal region and will accelerate the sequencing of a bipolar mood disorder

17 gene located at 18p.

18 The present invention provides, for the first time, a localization of a BP-I

19 susceptibility locus to a 300 to 500 kb region of the short arm of chromosome 18.

20 The present invention is directed to methods of detecting the presence of a bipolar

21 mood disorder susceptibility locus in an individual, comprising analyzing a sample of DNA

22 for the presence of a DNA polymorphism on the short arm of chromosome 18 between

23 SAVA5 and ga203, wherein the DNA polymorphism is associated with a form of bipolar

24 mood disorder. The invention includes the use of genetic markers in the roughly 500 kb

25 region between the SAVA5 locus and the ga203 locus, inclusive, to diagnose bipolar mood

26 disorder genetically in individuals and to confirm phenotypic diagnoses of bipolar mood

27 disorder. Preferably, the sample of DNA is analyzed for the presence of a DNA

28 polymorphism on the short arm of chromosome 18 in the roughly 300 kb region between

29 D18S1140 and W3422.

1   In a further embodiment, the invention provides methods of classifying subtypes of

2   bipolar mood disorder by identifying one of more DNA polymorphisms located within the

3   500 kb region between SAVA5 and ga203 loci, inclusive, on the short arm of chromosome

4   18 and analyzing DNA samples from individuals phenotypically diagnosed with bipolar mood

5   disorder for the presence or absence of one or more of said DNA polymorphisms.

6   Preferably, the sample of DNA is analyzed for the presence or absence of one or more of

7   said DNA polymorphisms in the roughly 300 kb region between D18S1140 and W3422 on

8   the short arm of chromosome 18.

9   In yet a further embodiment, the methods of the invention include a method of

10   treating an individual diagnosed with bipolar mood disorder comprising identifying one or

11   more DNA polymorphisms located within the 500 kb region of chromosome 18 between

12   SAVA5 and ga203, analyzing DNA samples from individuals phenotypically diagnosed with

13   bipolar mood disorder for the presence or absence of one or more of the DNA

14   polymorphisms, and selecting a treatment plan that is most effective for individuals having a

15   particular genotype within the 500 kb region of chromosome 18 between SAVA5 and ga203.

16   Preferably, the sample of DNA is analyzed for the presence or absence of one or more DNA

17   polymorphisms in the roughly 300 kb region between D18S1140 and W3422 on the short

18   arm of chromosome 18.

19

20   BRIEF DESCRIPTION OF THE DRAWINGS

21   FIG. 1 is a pedigree chart showing two families, CR001 and CR004. Affected

22   individuals are denoted by black symbols, deceased individuals by a diagonal slash. A

23   schematic of each individual's haplotype (where available) is shown below the ID number.

24   Recombinations are denoted by "-x"; consanguineous marriages by a double bar, and the

25   conserved haplotype as black shading within the haplotype bars. The larger conserved region

26   for CR004 is stippled, the larger conserved region for CR001 is indicated by a dashed

27   outline. An "I" underneath the haplotype bars indicates inferred haplotype. A "?" indicates

28   phase is uncertain. The connection between CR001 and CR004, dating to an 18th Century

29   founding couple, is indicated by the dashed lines joining individuals III-6 and I-4.

30

1   FIG. 2 is a table of lod scores for markers covering the entire human genome that

2   exceeded the arbitrary coverage thresholds. Lod scores are shown for two markers on

3   chromosome 18: D18S59 and D18S1105.

4

5   FIG. 3 depicts the extent of marker coverage used in the pedigree genome screening

6   study for each chromosome. Coverage is defined as regions for which a lod score of at least

7   1.6 would have been detected (in the combined data set) for markers truly linked to BP-I

8   under the model employed. Areas that remain uncovered (at this threshold) are unshaded.

9   Markers for which lod scores were obtained that exceeded the empirically determined

10   coverage thresholds in CR001, CR004, or the combined data set, are shown at their

11   approximate chromosomal location. The symbols to the right of the chromosome indicate the

12   thresholds exceeded at that marker: a circle signifies that the lod score at a marker exceeded

13   the threshold of 0.8 in CR001, a diamond signifies that the lod score exceeded the threshold

14   of 1.2 in CR004, and a star signifies that the lod score exceeded the threshold of 1.6 in the

15   combined data set.

16

17   FIGS. 4A and 4B depicts the Lod score for the maximum likelihood estimate of theta

18   in the combined sample for the 473 microsatellite markers typed in the pedigree genome

19   screen. The MLEs of theta were appointed to the following categories: theta < 0.10; 0.10

20   ≤ theta ≤ 0.40; theta ≥0.40. Note that the scale for the x-axis (distance from pter)

21   changes with chromosomes.

22

23   FIG. 5 is a portion of an integrated map of the 5 cM 18pter region of chromosome

24   18.

25

26   FIGS. 6A, 6B and 6C are a list of markers on chromosome 18, with map positions

27   noted.

28

29   FIG. 7 describes 18p allele frequencies for disease chromosomes (aff 105) versus

30   nontransmitted.chromosomes (ntrans) and samples from a control population of Costa Rican

1   students and their parents (control). The name of each marker used in this study is indicated

2   on the left. The second column of numbers refers to allele length in base pairs.

3

4   FIG. 8 depicts haplotype analysis of individuals affected with BP-I. The column

5   labelled 18p refers to the patient identifier, and each patient identifier is repeated with 2 rows

6   to indicate allele results with each of the patient's two copies of chromosome 18. The

7   columns labelled "PANR" and "MANR" refer to the paternal and maternal identifiers,

8   respectively, associated with the particular patient, other than 0, 1 and 2, which indicate that

9   parental samples were not available. The column headings to the right of "PANR" and

10   "MANR" columns represent names of specific markers in the 18p region that were used in

11   the haplotype analysis. The markers are listed in the order they appear on chromosome 18.

12   The allele length (in base pairs) is indicated under the column heading each marker for a

13   particular patient. In the column to the immediate right of each marker column, a "1"

14   indicates that the phase is known, i.e., that it is known whether a particular allele is inherited

15   from the paternal or maternal chromosome, and a "0" indicates that the phase is not

16   definitely known. The shaded horizontal bars depict haplotypes of at least three markers

17   which include a 154 allele length at D18S59, other than patients 218, 225, 232, 234, 311,

18   314 and 458, where the stippled region depicts small sections that do not have the 154 allele

19   at D18S59. The hatched regions depict uncertainty as to whether the individual has the

20   affected haplotype, as the phase is not known with certainty. In addition, the presence of an

21   allele length of 232 (or 234) with marker ta201 is thought to result from a highly mutable

22   allele and may not be distinct from the 230 allele. Similarly, the 202 allele at ca212 may not

23   be distinct from the 200 allele at ca212. Patients 246, 247, 248, 311, 316, 367, 384, 501,

24   531, 587, 536, 684, 667 and 669 exhibit a 242, 244, 250, 252 or 214 allele at marker ta201

25   which indicates a potential marker location. Patients 488, 435 and 236 exhibit haplotypes

26   that are distinct from the pedigrees that were analyzed.

27

28   FIG. 9 depicts haplotype analysis of nontransmitted chromosomes from parents of

29   individuals affected with BP-I. The labels "ERSN" and "KID" refer to the parental and

30   patient identifiers, respectively. As above, allele length is provided in base pairs below each

1   marker with an indication as to whether phase was known (1) or not known (0) given to the

2   right of these values. The markers, shading and allele characteristics described for Figure 8

3   also apply to this figure.

4

5

6   FIG. 10 depicts haplotype analysis of control samples obtained from an unscreened

7   population of students of the University of Costa Rica and their parents representing the

8   general population. Identifiers are provided in the column headed "cont", allele length and

9   phase determination given in the remainder of the table. The markers, shading and allele

10   characteristics described for Figure 8 also apply to this figure. Complete data for all

11   markers are not given as indicated by blank boxes, or the terms "miss" or "missing".

12

13   FIG. 11 depicts Ancestral Haplotype Reconstruction results in disease chromosomes.

14

15   DESCRIPTION OF SPECIFIC EMBODIMENTS

16   The recent availability of highly polymorphic, genetically mapped markers covering

17   the human genome (Weissenbach, J., et al. (1996) Nature 359, 794-801, Murray, J.C., et al.

18   (1994) Science 265, 2049-2054, Gyapay, G., et al. (1994) Nature Genet 7,246-339) has

19   allowed the development of a multi-stage paradigm for mapping genes for complex traits. In

20   the first stages, complete genome screening (e.g. through lod score analysis) is used to

21   identify possible localizations for disease genes. Subsequently, the regions highlighted by the

22   screening study are more intensively investigated to confirm the initial localizations and

23   delineate clear candidate regions. Finally, fine mapping methods (such as haplotype or

24   linkage disequilibrium (LD) analysis) or candidate gene approaches are used for positional

25   cloning of disease genes.

26   Our genome screening study for BP employed the following strategies. Unlike

27   previous genetic studies of BP, only those individuals with the most severe and clinically

28   distinctive forms of BP (BP-I and schizoaffective disorder manic type, SAD-M) were

29   considered as affected, rather than including those diagnosed with a milder form of BP (BP-

30   II) or with unipolar major depressive disorder (MDD). Two large pedigrees (CR001 and

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9.

1   CR004) were selected from a genetically homogeneous population, that of the Central Valley

2   of Costa Rica (as described in Escamilla, M.A., et al., (1996) Neuropsychiat. Genet. 67,

3   244-253, and in Freimer, N.B., et al. (1996) Neuropsychiat. Genet. 67, 254-263, both

4   incorporated by reference herein). The entire human genome was screened for linkage using

5   mapped microsatellite markers and a model for genetic analysis in which most of the linkage

6   information was derived from affected individuals. The goal of this stringent linkage

7   analysis was to identify all regions potentially harboring major genes for BP-I in the study

8   population. Empirically determined lod score thresholds (using linkage simulation analyses)

9   were derived, to suggest regions worthy of further investigation.

10   Identification of all suggestive regions and weighing the relative importance of

11   findings required complete screening of the genome. The coverage approach was developed

12   to gauge the progress of this effort. Conventionally, the thoroughness of genome screening

13   is evaluated by excluding genome regions from linkage under given genetic models. This

14   approach, which is highly sensitive to misspecification of genetic models, may be poorly

15   suited for genome screening studies of complex traits; it is tied to the expectation of finding

16   linkage at a single locus and demonstrating absence of linkage at all other locations in the

17   genome. Additionally, exclusion analyses do not differentiate between genome regions

18   where linkage is not excluded because markers are uninformative in the study population

19   from those in which the genotype data are simply ambiguous. In contrast, the coverage

20   approach is designed for studies aimed at genome screening rather than for studies where the

21   goal is to demonstrate a single unequivocal linkage finding, and it provides explicit data

22   regarding the informativeness of markers in the study pedigrees. Its use lessens the

23   possibility that one would prematurely dismiss a given genome region as being unpromising

24   for further study.

25   Because the exact genetic length of chromosomes is not clearly established, it is

26   impossible to be certain that one has screened the entire genome. Although we report

27   coverage of about 94% of the genome (under the 90%) dominant model) at the thresholds

28   described above, this probably represents an underestimate. The remaining coverage gaps in

29   our study occur predominantly at or near telomeres; as the upper bound estimates for the

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10.

1 length of each chromosome were used, it is likely that the actual coverage gaps in these

2 regions are smaller than our conservative assessment.

3   The presence of consistently positive lod scores over a given region was considered to

4 be of greater significance than isolated peak lod scores. Such clustering suggests true co-

5 segregation of markers and phenotypes (i.e. alleles are shared identically by descent rather

6 than identically by state) and is more readily observed in analyses of a few large pedigrees

7 (as in our study) than in examination of several smaller families. The data presented herein

8 indicates clustering of positive lod scores in the region of the telomere of 18p.

9   The genome screen was conducted in two stages. The Stage I screen identified areas

10 suggestive of linkage, so that those areas could be saturated with available markers, and so

11 that regions, referred to as 'coverage gaps', could be pinpointed where markers were

12 insufficiently informative in our sample to detect evidence of linkage. The Stage II screen

13 followed up on regions flanking each marker that yielded peak lod scores approximately

14 equal to or greater than the thresholds used for the coverage calculations, which were

15 deemed regions of interest, and filled in coverage gaps. The results of the complete genome

16 screen (Stages I and II) using 473 markers is described below.

17   In addition, linkage disequilibrium analysis of an independently collected sample of 48

18 unrelated BP-I patients was initially conducted. These patients were from the same ancestral

19 population as the patients in the CR001 and CR004 pedigrees. The LD analysis was

20 conducted with markers on the short arm of chromosome 18 (18p), in a 5 centimorgan (cM)

21 region ("5 cM 18pter region") extending from the end of the 18p telomere to a distance of 5

22 cM along the short arm of chromosome 18 (18p). The LD analysis gave evidence of LD in

23 this region, particularly at marker D18S59 and also at D18S476. LD analysis of further BP-

24 I patients from the CRCV with markers in this 5 cM 18pter region was conducted to confirm

25 and fine map a BP-I gene in this region. This approach, using additional BP-I patients from

26 this CRCV population and additional markers identifies the region of maximum LD and can

27 precisely localize a BP-I susceptibility gene.

28   Fine mapping of 5 cM 18pter region resulted in the identification of two DNA

29 markers (D18S1140 and W3422) defining the boundaries of BP-I as approximately 300 kb,

30 thus allowing a systematic search for the BP-I gene(s).

1   A conservative approach to linkage analysis was used in that almost all of the

2 information for linkage is derived from individuals with a severe, narrowly defined

3 phenotype. While this approach made it very unlikely that lod scores greater than

4 conventional thresholds of statistical significance (e.g. ≥ 3) would be obtained, it provided

5 confidence in the robustness of the most suggestive findings.

6   Direct cDNA selection can be used to isolate segments of expressed DNA from the

7 300 kb region between D18S1140 and W3422 (M. Lovett, J. Kere, L.M. Hinton, *Proc.*

8 *Natl. Acad. Sci. USA* 88 9628-9632 (1991); Y.-S. Jou *et al., Genomics* 24 410-413 (1994)).

9 By using bacterial artificial chromosomes (BAC) (e.g., commercially available from

10 Research Genetics Inc. Huntsville, Alabama), a group of cDNAs can be identified, and

11 hybridization and PCR-amplification experiments can be used to determine if these cDNA

12 segments are derived from the 300 kb region.

13   The cDNAs can then be used to determine whether specific sequences are expressed

14 at lower levels (or not at all) in affected individuals compared to non-carrier individuals.

15 Measurement of mRNA levels in lymphoblastoid cell lines can be used as an initial screen.

16 The cell lines are prepared by drawing blood from individuals, transforming the lymphoblasts

17 with EBV and growing the immortalized cells in culture. Total RNA and DNA are extracted

18 from the cultured human lymphoblastoid cell lines. Northern blot hybridization is used to

19 determine reduced levels of a specific sequence compared to levels from an unaffected, non-

20 carrier individual as a result of mutations in the BP-I gene on the chromosomes from these

21 affected individuals which results in decreased levels of mature mRNA and play a primary

22 role in BP-I. Thus, alterations in gene sequences in affected individuals can be determined.

23   The polymerase chain reaction (PCR) is used to amplify the gene and to determine its

24 sequence from affected individuals. Sequence comparison with unaffected, non-carrier

25 individuals is carried out to identify polymorphisms in the gene sequence that are responsible

26 for BP-I.

27   The identification of the biochemical defect that causes BP-I provides a basis for

28 treatments for this disease. In addition, knowledge that certain mutations in the gene are

29 responsible for the disease allows mutation detection tests to be used as a definitive diagnosis

30 for BP-I.

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1   Thus, the present invention allows the isolation of a nucleic acid molecule that can be

2 used in the identification of the presence (or absence) of a mutation in the BP-I gene a human

3 and thus can be used in the diagnosis of BP-I or in the genetic counseling of individuals, for

4 example those with a family history of BP-I (although the general population can be screened

5 as well). In particular, it should be noted that any mutation in the BP-I gene away from the

6 normal gene sequence is an indication of a potential genetic flaw; even so-called "silent"

7 mutations that do not encode a different amino acid at the location of the mutation are

8 potential disease mutations, since such mutations can introduce into (or remove from) the

9 gene an untranslated genetic signal that interferes with the transcription or translation of the

10 gene. Thus, advice can be given to a patient concerning the potential for transmission of BP-

11 I if any mutation is present. While an offspring with the mutation in question may or may

12 not have symptoms of BP-I, patient care and monitoring can be selected that will be

13 appropriate for the potential presence of the disease; such additional care and/or monitoring

14 can be eliminated (along with the concurrent costs) if there are no differences from the

15 normal gene sequence. As additional information (if any) becomes available (e.g., that a

16 given silent mutation or conservative replacement mutation does or does not result in BP-I),

17 the advice given for a particular mutation may change. However, the change in advice given

18 does not alter the initial determination of the presence or absence of mutations in the gene

19 causing BP-I.

20   Generally, mutations are identified in the human gene for use in a method of detecting

21 the presence of a genetic defect that causes or may cause BP-I, or that can or may transmit

22 BP-I to an offspring of the human. Initially, the practitioner will be looking simply for

23 differences from the sequence identified as being normal and not associated with disease,

24 since any deviation from this sequence has the potential of causing disease, which is a

25 sufficient basis for initial diagnosis, particularly if the different (but still unconfirmed) gene

26 is found in a person with a family history of BP-I. As specific mutations are identified as

27 being positively correlated with BP-I (or its absence), practitioners will in some cases focus

28 on identifying one or more specific mutations of the gene that changes the sequence of a

29 protein product of the gene or that results in the gene not being transcribed or translated.

30 However, simple identification of the presence or absence of any mutation in the gene of a

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13.

1 patient will continue to be a viable part of genetic analysis for diagnosis, therapy and
2 counseling.

3 The actual technique used to identify the gene or gene mutant is not itself part of the
4 practice of the invention. Any of the many techniques to identify gene mutations, whether
5 now known or later developed, can be used, such as direct sequencing of the gene from
6 affected individuals, hybridization with specific probes, which includes the technique known
7 as allele-specific oligonucleotide hybridization, either without amplification or after
8 amplification of the region being detected, such as by PCR. Other analysis techniques
9 include single-strand conformation polymorphism (SSCP), restriction fragment length
10 polymorphism (RFLP), enzymatic mismatch cleavage techniques and transcription/translation
11 analysis. All of these techniques are described in a number of patents and other publications;
12 see, for example, "Laboratory Protocols for Mutation Detection" (1996) Oxford University
13 Press, Editor: Ulf Landegrun.

14 Depending on the patient being tested, different identification techniques can be
15 selected to achieve particularly advantageous results. For example, for a group of patients
16 known to be associated with particular mutations of the gene, oligonucleotide ligation assays,
17 "mini-sequencing" or allele-specific oligonucleotide (ASO) hybridization can be used. For
18 screening of individuals who are not known to be associated with a particular mutation,
19 single-strand conformation polymorphism, total sequencing of genetic and/or cDNA and
20 comparison with standard sequences are preferred.

21 In many identification techniques, some amplification of the host genomic DNA (or of
22 messenger RNA) will take place to provide for greater sensitivity of analysis. In such cases
23 it is not necessary to amplify the entire gene, merely the part of the gene or the specific
24 location within the gene that is being detected. Thus, the method of the invention generally
25 comprises amplification (such as via PCR) of at least a segment of the gene, with the
26 segment being selected for the particular analysis being conducted by the diagnostician.

27 The patient on whom diagnosis is being carried out can be an adult, as is usually the
28 case for genetic counseling, or a newborn, or prenatal diagnosis can be carried out on a
29 fetus. Blood samples are usually used for genetic analysis of adults or newborns (e.g.,

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14.

1 screening of dried blood on filter paper), while samples for prenatal diagnosis are usually

2 obtained by amniocentesis or chorionic villus biopsy.

3   Prior to the present invention, affected individuals were prescribed one drug after

4 another until one was found to be effective. As BP-I was diagnosed using clinical criteria,

5 no correlation between using a particular drug and its efficacy in a given case was observed.

6 As a result of the present invention, BP-I subtypes can be diagnosed at the molecular level

7 and effective treatment predicted.

8   For example, lithium salts, carbamazepine and valproic acid have all been prescribed

9 for BP-I affected individuals with serendipitous results. An individual can now be diagnosed

10 with bipolar mood disorder by analyzing genetic material from that individual for the

11 presence or absence of one or more nucleic acid mutations as described above. As a result

12 of this diagnosis at the molecular level, an effective treatment can be determined by

13 collecting data to obtain a statistically significant correlation of a particular treatment with

14 the different subtypes of BP-I. Thus, the practitioner is able to select a specific drug for the

15 treatment of a particular sub-type of BP-I and does not merely rely on trial and error.

16   Alternatively, the full-length normal genes for BP-I from humans, as well as shorter

17 genes that produce functional proteins, can be used to correct BP-I in a human patient by

18 supplying to the human an effective amount of a gene product of the human gene, either by

19 gene therapy or by *in vitro* production of the protein followed by administration of the

20 protein. It should be recognized that the various techniques for administering genetic

21 materials or gene products are well known and are not themselves part of the invention. The

22 invention merely involves supplying the genetic materials or proteins identified as a result of

23 the present invention in place of the genetic materials or proteins previously administered.

24 For example, techniques for transforming cells to produce gene products are described in

25 U.S. Patent No. 5,283,185 entitled "Method for Delivering Nucleic Acid into Cells," as well

26 as in numerous scientific articles, such as Felgner et al., "Lipofection: A Highly Efficient,

27 Lipid-Mediated DNA-Transfection Procedure," *Proc. Natl. Acad. Sci. U.S.A.*, 84, 7413-

28 7417 (1987); techniques for in vivo protein production are described in, for example,

29 Mueller et al., "Laboratory Methods - Efficient Transfection and Expression of Heterologous

30 Genes in PC12 Cells," *DNA and Cell Biol.*, 9(3), 221-229 (1990).

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15.

1   Administration of proteins and other molecules to overcome a deficiency disease is

2   well known (e.g., administration of insulin to correct for high blood sugar in diabetes) that

3   further discussion of this technique is not necessary. Some modification of existing

4   techniques may be required for particular applications, but those modifications are within the

5   skill level of the ordinary practitioner using existing knowledge and the guidance provided in

6   this specification.

7   The invention now being generally described, the following examples are provided for

8   purposes of illustration only and are not to be considered to limit the invention.

9

10

11   EXAMPLES

12   PEDIGREES

13   Two independently ascertained Costa Rican pedigrees (CR001 and CR004) were

14   chosen because they contained a high density of individuals with BP-I and because their

15   ancestry could be traced to the founding population of the Central Valley of Costa Rica. The

16   current population of the Central Valley (consisting of about two million people) is

17   predominantly descended from a small number of Spanish and Amerindian founders in the

18   16th and 17th centuries (Escamilla, M.A., et al., (1996) Neuropsychiat. Genet. 67, 244-

19   253). Studies of several inherited diseases have confirmed the genetic isolation of this

20   population (Leon, P., et al. (1992) Proc. Natl. Acad. Sci. USA. 89, 5181-5184;

21   Uhrhammer, N., et al. (1992) Am. J. Hum. Genet. 57, 103-111). An extensive description

22   of pedigrees CR001 and CR004 has ben published (Freimer, N.B., et al. (1996)

23   Neuropsychiat. Genet. 67, 254-263). In the course of the study, two links between these

24   pedigrees were discovered. However, the families were analyzed separately because these

25   links were discovered after the simulation analyses were completed and after the genome

26   screening study had been initiated.

27   All available adult members of these families were interviewed in Spanish using the

28   Schedule for Affective Disorders and Schizophrenia Lifetime version (SADS-L) (Endicott, J.

29   et al, (1978) Arch. Gen. Psych. 35, 837-844). Individuals who received a psychiatric

30   diagnosis were interviewed again in Spanish by a research psychiatrist using the Diagnostic

1 Interview for Genetic Studies (DIGS) (Nurnberger, J.L. et al. (1994) Arch. Gen. Psychiat.

2 51, 849-859). This recently developed instrument is similar to, but more detailed than

3 SADS-L. The interviews and medical records were then reviewed by two blinded best

4 estimators who reached a consensus diagnosis. The diagnostic procedures are described in

5 detail in Freimer, N.B., et al. (1996) Neuropsychiat. Genet. 67, 254-263 (incorporated by

6 reference herein).

7

8   UNRELATED CRCV BP-I PATIENT STUDY

9   BP localizations obtained through the CRCV pedigree studies were confirmed by

10 genotyping an independently collected sample of 48 unrelated BP-I patients from the CRCV.

11 In this fine mapping LD analysis, 48 unrelated BP-I patients from the CRCV were identified

12 and genotyped using microsatellite markers spaced at narrow intervals across chromosome

13 18. As these patients are descended from the same ancestral population as the patients in the

14 pedigrees previously studied (CR001 and CR004), many of them should share disease

15 susceptibility alleles inherited identically by descent (IBD) from one or a few common

16 ancestors, and linkage disequilibrium (LD) should be present at marker loci surrounding the

17 disease genes.

18   The sample of 48 BP-I patients included 25 women and 23 men who were recruited

19 from psychiatric hospitals and clinics in the CRCV. These patients were ascertained only on

20 the basis of diagnosis and CV ancestry, and were not selected on the basis of history of BP

21 illness in family members. A structured interview of each patient was conducted by a

22 psychiatrist, and medical and hospital records were collected. Ascertainment and diagnostic

23 procedures were as described above. However, in order to lessen further the probability of

24 phenocopies among this unrelated sample, for which we lacked pedigree information, the

25 affected phenotype was defined even more narrowly than in the pedigree study. Individuals

26 considered affected in this study had to have suffered at least two disabling episodes of mania

27 (requiring hospitalization) and a first onset of the illness before age 45.

28   Genealogical research on each of the 48 BP-I patients confirmed that on average, 70%

29 of their great-grandparents were born in the CRCV. Individuals whose great-grandparents

30 were born in the CRCV were considered likely to be descended from the original Spanish

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1   and Amerindian founders of the CRCV. Genealogical research showed that 2 patients are

2   first cousins and the remaining 46 have no relationship within the past 4 generations.

3

4   GENOTYPING PEDIGREE STUDIES

5   Linkage simulations were used to select the most informative individuals from

6   pedigrees CR001 and CR004 for genotyping studies (Freimer, N.B., et al. (1996)

7   Neuropsychiat. Genet. 67, 254-263). Under a 90% dominant model, simulation analyses

8   with these individuals suggested that evidence of linkage would likely be detected (e.g. a

9   probability of 92% of obtaining lod > 1.0 in the combined data set) using markers with an

10   average heterozygosity of 0.75 spaced at 10 cM intervals (as discussed in Freimer, N.B., et

11   al. (1996) Neuropsychiat. Genet. 67, 254-263). For the Stage I screen, the most

12   polymorphic markers (307 in total) were chosen, placed at approximately 10 cM intervals on

13   the 1992 Genethon map (Houwen, R., et al. (1992) Nature 359, 794-801). These markers

14   were then supplemented by a small number of markers from the Cooperative Human Linkage

15   Center (CHLC) public database. For the Stage II screen, 166 markers were added from

16   newer Genethon and CHLC maps as they became available (Murray, J.C. et al. (1994)

17   Science 265, 2049-2054, Gyapay, G., et al. (1994) Nature Genet. 7,246-339) and from the

18   public database of the Utah Center for Genome Research, all of which are publicly available.

19   DNA samples (from individuals in the CEPH families) that were used for size standards for

20   Genethon and CHLC markers were included in the experiments to permit comparison of

21   allele sizes between members of the CRCV population and individuals in the CEPH database.

22   Genotyping procedures were as described previously (DiRienzo, A. et al. (1994) Proc. Natl.

23   Acad. Sci. USA 91, 3166-3170 (incorporated by reference herein)). Briefly, one of the two

24   PCR primers was labeled radioactively using a polynucleotide kinase and PCR products were

25   run on polyacrylamide gels. Autoradiographs were scored independently by two raters.

26   Data for each marker were entered into the computer database twice and the resultant files

27   were compared for discrepancies.

28

GENOTYPING OF UNRELATED BP-I CRCV PATIENTS

1

2   Twenty-seven markers were used to genotype all 48 individuals (as well as 53

3   individuals used to establish genetic phase) at approximately 5 cM intervals along the entire

4   chromosome 18. It was hypothesized that such a screen would permit the evaluation of

5   evidence in the 18pter region and also to investigate other regions on chromosome 18 in

6   which linkage to BP has been suggested by other groups in other populations. For each

7   individual, two-marker haplotypes in each of the 26 inter-marker intervals were investigated.

8   For 38 of the 48 BP-I patients, genotypes of parents or children were available to assist in

9   phase determination. Because of phase ambiguities in the remaining 10 individuals, minimal

10 and maximal two-marker haplotype sharing was evaluated as follows: (1) Minimal: the

11 number of individuals (and chromosomes) who definitely shared a chromosomal segment

12 defined by a particular pair of alleles (phase known chromosomes) and (2) Maximal: the

13 number of individuals (and chromosomes) who could possibly share a chromosomal segment

14 defined by a particular pair of alleles (includes phase unknown chromosomes). The threshold

15 used to identify areas of high IBD sharing of chromosomes in this initial screen was

16 designated as maximal sharing of a two-marker haplotype by 50% or more of the 48

17 individuals (or 25% or more of the 96 chromosomes).

18   Arbitrary thresholds were designated to identify possible areas of high IBD sharing

19 among the 48 patients. Eight of the 26 regions passed this screen. Within each of these 3

20 regions, one to three additional markers were typed to permit detection of LD, if present,

21 over regions of one to two cM.

22   A total of 42 chromosome 18 markers were used to genotype the study sample:

23 D18S1140, D18S59, D18S476, D18S481, D18S391, D18S452, D18S843, D18S464,

24 D18S1153, D18S378, D18S53, D18S453, D18S40, D18S66, D18S56, D18S57, D18S467,

25 D18S460, D18S450, D18S474, D18S69, D18S64, D18S1134, D18S1147, D18S60, D18S68,

26 D18S55, D18S477, D18S61, D18S488, D18S485, D18S541, D18S870, D18S469, D18S874,

27 D18S380, D18S1121, D18S1009, D18S844, D18S554, D18S461, D18S70 (from pter to

28 qter). Of these 42 markers, four are located within the 5 cM 18pter region extending from

29 the telomere of 18p to marker D18S481 (inclusive), which is approximately 5 cM from the

1   telomere of 18p. This region is referred to as the 5 cM 18pter region. The four markers

2   tested in the 5 cM 18pter region are: D18S59, D18S1140, D18S476 and D18S481.

3   For each marker the likelihood that a particular allele (or alleles) is over-represented

4   on disease chromosomes, as compared to non-disease chromosomes was evaluated. The

5   results of this likelihood test provide a conservative but powerful measure of LD between

6   two loci.

7

8   **PEDIGREE STATISTICAL ANALYSES**

9   Two-point linkage analyses were performed for all markers. Marker allele

10 frequencies were estimated from the combined data set with correction for dependency due to

11 family relationships (Boehnke, M. (1991) Am. J. Hum. Genet. 48, 22-25). The linkage

12 analyses for Stages I and II included the 65 individuals who were genotyped as well as an

13 additional 65 individuals who had been diagnostically evaluated but not genotyped. Only

14 individuals with BP-I were considered affected with the exception of two persons, one in

15 each family, who carry diagnoses of schizoaffective disorder manic type (SAD-M). The

16 SAD-M individuals were included as affected because BP-I and SAD-M are often difficult to

17 distinguish from each other based on their clinical presentation and course of illness

18 (Goodwin, F.K. et al. (1990) in Manic Depressive Illness (Oxford University Press, New

19 York), pp. 373-401; Freimer, N.B et al. (1993) in The Molecular and Genetic Basis of

20 Neurological Disease, pp. 951-965; Freimer, N.B. et al. (1996) Neuropsychiat. Genet. 67,

21 254-263; and Freimer, N.B. et al (1996) Nature Genetics 12:436-441, all incorporated by

22 reference herein). In all, 20 individuals were designated as affected within CR004

23 (Copeman, J.B., et al. (1995) Nature Genet. 9, 80-85 available for genotyping) and

24 10 individuals from CR001 (Kelsoe, J.R. et al. (1989) Nature 342, 238-243 available for

25 genotyping). The phenotype for all other individuals was designated as unknown except for

26 17 individuals who were designated as unaffected because they had been thoroughly clinically

27 evaluated, showed no evidence of any psychiatric disorder, and were well beyond the age of

28 risk (50) for BP-I (linkage simulation studies indicated that these unaffected individuals

29 contributed little information to the linkage analysis).

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20.

1   Linkage analyses were performed using a nearly dominant model (assuming

2   penetrance of 0.81 for heterozygous individuals of 0.9 for homozygotes with the disease

3   mutation). This model was chosen from five different single-locus models (ranging from

4   recessive to nearly dominant) due to its consistency with the segregation patterns of BP in the

5   two pedigrees and because it had demonstrated the greatest power to detect linkage in

6   simulation studies (Freimer, N.B., et al. (1996) Neuropsychiat. Genet. 67, 254-263). Based

7   on Costa Rican epidemiological surveys Escamilla, M.A., et al., (1996) Neuropsychiat.

8   Genet. 67, 244-253, the population prevalence of BP-I was assumed to be 0.015 (and thus

9   the frequency of the disease allele was assumed to be 0.003)(based on epidemiological

10   surveys in Costa Rica, Adis, G. (1992) "Disordenes mentales en Costa Rica: Observaciones

11   Epidemiologicas," (San Jose, Costa Rica: Editorial Nacional de Salud y Seguridad Social)).

12   The frequency of BP-I in individuals without the disease allele was conservatively set at 0.01

13   which effectively specified a population phenocopy rate of 0.67 (i.e., an affected individual

14   in the general population has a 2/3 probability of being a phenocopy). For multiply affected

15   families, the probability that a gene segregates is highly increased, which implies that

16   affected individuals in our study pedigree have a lower probability to be phenocopies than

17   affected individuals in the general population, particularly those with several affected close

18   relatives (the exact probabilities are dependent on the degree of relationship between patients

19   and the number of intervening unaffected individuals). These parameters were chosen to

20   ensure that most of the linkage information derives from affected individuals. The rationale

21   for selecting these parameters and results of analyses that demonstrate the conservatism of

22   this model are described by Freimer, N.B., et al. (1996) Neuropsychiat. Genet. 67, 254-263.

23   The LINKAGE package (Lathrop et al., (1984) Proc. Natl. Acad. Sci. USA 81, 3443-3446)

24   was used for lod score analysis and to obtain maximum likelihood estimates of the marker

25   allele frequencies, taking into account the existing family relationships (see Boehnke, Am. J.

26   Hum. Gent. 48, 22-25 (1991)).

27

28   **UNRELATED BP-I CRCV PATIENT STATISTICAL ANALYSES**

29   A likelihood test of disequilibrium (J. Terwilliger, Am. J. Hum. Genet. 56, 777

30   (1995)) was used to estimate a single parameter, lambda, that quantifies the over-

1 representation of marker alleles on disease chromosomes as compared to non-disease

2 chromosomes. We chose this method of analysis over another commonly used

3 disequilibrium analysis method, the transmission disequilibrium test (TDT, R. Spielman et

4 al., Am. J. Hum. Genet. 52, 506 (1993)) because data from all 48 BP-I patients could be

5 used in the likelihood approach. Effective use of the TDT requires phase-known,

6 heterozygous parental chromosomes. We do not have parental genotypes for 20 of the 48

7 BP-I patients. Simulations indicated that with our data, the likelihood test of disequilibrium

8 would be more powerful than the TDT. Lambda has been shown to be a superior measure

9 for LD fine mapping, compared to other frequently used measures, because it is directly

10 related to the recombination fraction between the disease and the marker loci. Non-disease

11 chromosomes were chosen from the phase-known chromosomes of parents, spouses and

12 children of affected individuals, if available. Designation of chromosomes of family

13 members as non-disease in a disorder such as BP-I, which is not fully penetrant, necessitates

14 specifying a model of disease transmission. The same model of transmission was employed

15 in this LD likelihood test as was used in the initial genome screen of the pedigrees CR001

16 and CR002 described herein. One parameter was specified differently from the genome

17 screen: the phenocopy rate was set to zero in the LD likelihood analysis. A phenocopy rate

18 was not specified in the transmission model because the effect of phenocopies will be

19 "absorbed" by the lambda parameter, in that presence of phenocopies in our sample will

20 serve to erode the association between marker alleles and disease, and hence reduce the

21 estimate of lambda.

22

23 COVERAGE

24 To access coverage for a marker, the number of informative meioses at the estimated

25 recombination fraction was calculated using the estimate of the variance (the inverse of the

26 information matrix) (Petrukhin, K.E. et al. (1993) Genomics 15, 76-85). Alternatively,

27 when the estimated frequency of recombination was close to 0 or 1, Edwards' equation was

28 applied to calculate the equivalent number of observations (Edwards, J.H. (1971) Ann. Hum.

29 Genet. 34, 229-250). These meioses represent the amount of linkage information provided

30 by the marker,. given the pedigree structure and the genetic model applied. Linkage to the

1   marker in question was then assumed and the lod score that would be observed as a disease

2   gene is hypothetically moved in increments away from that marker was calculated. All

3   regions around a marker that would have generated a lod score that exceeded our thresholds

4   for possible linkage (0.8 in CR001, 1.2 in CR004, and 1.6 in the combined data) were

5   considered covered. These lod score thresholds were derived from simulation analyses

6   showing the expected distribution of lod scores under linkage and non-linkage (Freimer,

7   N.B., et al. (1996) Neuropsychiat. Genet. 67, 254-263, and approximately represent a result

8   that is 250 times more likely to occur in linked simulations than in unlinked simulations.

9   Coverage maps were constructed (FIG. 1) by superimposing the regions covered by each

10   marker on the genetic map of each chromosome. At the end of the Stage II screen, a total of

11   473 microsatellite markers had been typed with genome coverage (in the combined data set)

12   of over 94%. Possible coverage gaps are indicated by unshaded areas and are mainly

13   concentrated near telomeres. Because the coverage calculations make use of marker

14   informativeness within the pedigrees, the coverage approach thus permits detection of

15   instances where markers with expected high heterozygosities are uninformative in our data

16   set.

17

18   PEDIGREE LINKAGE ANALYSIS RESULTS

19   Of the 473 microsatellites analyzed with two-point linkage tests, 23 markers exceeded

20   the empirically determined thresholds designated for the coverage calculations (in either

21   CR001, CR004, or in the combined data set). The location of these markers, the peak lod

22   scores obtained in each family and in the combined data set, and the maximum likelihood

23   estimate of the recombination fraction (0) at which these lod scores were observed are

24   indicated in **Table 1**. The approximate chromosomal locations of these markers are also

25   depicted in **FIG. 1**. The distribution of lod scores (for the maximum likelihood estimate of 0

26   in the combined data set) across the genome is displayed by chromosome in **FIG. 2**.

27   The threshold was exceeded for pedigree CR001 in two adjacent markers near the 18p

28   telomere (D18S59 and D18S1105), but CR004 displayed no suggestion of linkage in this

29   region.

1   In the genome screen, the highest lod score observed for family CR001 alone was at

2   D18S59 (1.32 at $\theta=0.0$), located near pter. All affected members of CR001 shared alleles at

3   markers in the 18pter region.

4

5   UNRELATED BP-I CRCV PATIENT STUDY RESULTS

6   Out of the forty-two markers tested, eight displayed evidence of over-representation

7   of a particular allele on disease chromosomes. Eight of the 42 markers had -2*ln(likelihood

8   ratio) statistics >1.0. Three other markers had -2*ln(likelihood ratio) statistics >0 and

9   <0.62. The results are shown in Table I:

10   Table I

Marker	Allele Size	Frequency on non-disease Chromosomes	Frequency on Disease Chromosomes
D18S59	154	0.121	0.572
D18S476	271	0.470	0.771
D18S467	172	0.384	0.693
D18S61	177	0.074	0.326
D18S485	182	0.237	0.586
D18S870	179	0.405	0.657
D18S469	234	0.128	0.450
D18S1121	168	0.171	0.553

24   Evidence for association was found at markers located near the telomere of the short

25   arm of chromosome 18. D18S59 displayed the strongest evidence for LD (-2*ln(likelihood

26   ratio) of 8.3, p=0.002) of all the chromosome 18 markers tested. An adjacent marker,

27   D18S476 (-2*ln(likelihood ratio) of 1.3), also provided evidence of LD. In our genome

28   screening pedigree study we observed the single highest lod score for pedigree CR001 of any

29   marker in the entire genome at D18S59. Furthermore, the alleles at D18S59 and D18S476

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24.

1   that are over-represented among the BP-I patients from the population sample (154 b.p. and

2   271 b.p. respectively) are observed in all BP-I patients from pedigree CR001.

3   The LD and pedigree findings in the 5 cM 18pter region denote a clearly delineated

4   region that contains a BP-I susceptibility locus. This region is distinct from other regions on

5   chromosome 18 that have been suggested as linked to mood disorder phenotypes (more

6   broadly defined than BP-I). See **FIG. 6A, 6B, 6C.** In contrast to previous reports by

7   Berrettini et al. and Stine et al., suggesting possible linkage between mood disorder and

8   markers in the pericentromeric region of chromosome 18, our results did not show any

9   evidence for association of BP-I with any pericentromeric markers (D18S378, D18S53,

10   D18S453 or D18S40).

11

12   IDENTIFICATION OF NEW MARKERS FROM THE 5 CM 18PTER REGION

13   Cloned human genomic DNA covering the target region is assembled. Microsatellite

14   sequences from these clones are identified. A sufficient area around the repeat to enable

15   development of a PCR assay for genomic DNA is sequenced, and it is confirmed that the

16   microsatellite sequence is polymorphic, as several uninformative microsatellites are expected

17   in any set. Several methods have been routinely used to identify microsatellites from cloned

18   DNA, and at this time no single one is clearly preferable (Weber, 1990, Hudson et al.,

19   1992). Most of these require screening an excessive number of small insert clones or

20   performing extensive subcloning using clones with larger inserts.

21   New strategies have recently been developed which permit the use of the several

22   different microsatellites to be found within a single large insert clone without requiring

23   extensive subcloning. A method for direct identification of microsatellites from yeast

24   artificial chromosomes (YACs) provides several new markers from the target region. This

25   procedure is based on a subtractive hybridization step that permits separation of the target

26   DNA from the vector background. This step is useful because the human DNA (the YAC)

27   constitutes only a small proportion of the total yeast genomic DNA.

28   YAC clones (with inserts averaging about 750 Kb of human genomic DNA) that span

29   the 5 cM 18pter region have already been identified by the CEPH/Généthon consortium

30   (Cohen et al., 1993) and are publicly available. The markers from YACs that have been

1 mapped to portions of the candidate region that are not well represented by currently

2 available markers are first isolated. By typing these markers in the families and the "LD"

3 sample, as described above, it is possible to narrow the candidate region, perhaps to a size of

4 less than one to two cM, thus permitting limitation of the segment in which more extensive

5 mapping efforts are applied.

6   Briefly, the microsatellite identification procedure is performed as follows: A

7 subtractive hybridization is performed using genomic DNA from a target YAC together with

8 an equivalent amount of a control DNA. This procedure separates the YAC DNA from that

9 of the yeast vector. Following the subtraction procedure the subtracted YAC DNA is

10 purified, digested with restriction enzymes and cloned into a plasmid vector (Ostrander et al.,

11 1992). The cloned products of each YAC are screened using a CA(15) oligonucleotide probe

12 (i.e. an oligonucleotide having 15 CA repeats). Each positive clone (i.e. those that contain

13 TG-repeats) is sequenced to identify primers for PCR to genotype the BP-I samples.

14   An alternative approach, based on using a set of degenerate sequencing primers that

15 anneal directly to the repeat sequence, permitting direct thermal cycle sequencing (Browne &

16 Litt, 1992), can also be used.

17   Once the candidate region is narrowed to a size of less than about 500 to 1000 Kb, a

18 contiguous array (contig) of clones with smaller inserts than YACs, mainly P1 clones, is

19 developed. P1 clones are phage clones specially designed to accommodate inserts of up to

20 100 Kb (Shepherd et al., 1994).

21

22 **DEVELOPMENT OF A PHYSICAL MAP OF THE 5 CM 18PTER REGION**

23   In parallel with the genetic mapping, a physical map of the 5 cM 18pter region is

24 developed. The backbone of this effort is the assembly of contigs of large insert clones.

25 Low resolution contigs for most of the human genome are already available using the YACs

26 developed by CEPH (Cohen et al., 1993). Although these have been individually verified

27 and checked for overlap with other YACs, there is a high rate of chimerism in the YACs and

28 insufficient evidence to definitively confirm the order of the YACs. In addition, because of

29 their large size these YACs are particularly cumbersome to work with. Nevertheless, they

30 provide a useful framework to start constructing high resolution contigs.

1   Once a candidate region of less than about five cM is delineated, the studies to

2   develop a physical map are commenced. Because of the disadvantages of relying solely on

3   YACs, and because positional cloning is facilitated by the availability of a higher resolution

4   map, contigs are generated using P1 clones once the candidate region is narrowed to less

5   than one Mb, by LD mapping in the expanded population sample using the new markers

6   identified from the YACs.

7   Once a region of 500-1000 Kb or less is defined, physical mapping and cloning are

8   computed using P1 clones rather than YACs, and P1 contigs over such a region are

9   constructed. The P1s are used to identify additional markers for the further positional

10   cloning steps as well as the screening for rearrangements.

11   The starting point of contig construction is the microsatellite sequences and non-

12   polymorphic STSs that derive from the few YACs that surround the genetically determined

13   candidate region. These STSs are used to screen the P1 library. The ends of the P1s are

14   cloned using inverse PCR and used to order the P1s relative to each other. Amplification in

15   a new P1 will indicate that it overlaps with the previous one. Fluorescent in situ

16   hybridization (FISH) permits ordering of the majority of the P1s (Pinkel, 1988; Lichter,

17   1991). The original set of P1s serves as building blocks of the complete contig; each end

18   clone is used to re-screen the library and in this way P1s are added to the map.

19   From each P1 additional microsatellites are identified as previously described. This

20   allows further reduction of the candidate region. When the region is narrowed to less than

21   one Mb in size, positional cloning efforts are initiated.

22   FINE MAPPING OF 5CM 18PTER REGION

23   In order to delineate further regions of BP-I susceptibility within the 5 cM 18pter

24   region, additional unrelated BP-I patients from the CRCV as well as other populations can be

25   diagnosed and genotyped both with the markers described herein as well as additional

26   markers in the 5 cM 18pter region that are known as well those yet to be identified.

27   Additional markers are available from the Cooperative Human Linkage Center (CHLC)

28   public database, from newer Genethon and CHLC maps as they become available (Murray,

29   J.C. et al. (1994) Science 265, 2049-2054, Gyapay, G., et al. (1994) Nature Genet. 7,246-

30   339) and from the public database of the Utah Center for Genome Research (all of which are

1   incorporated by reference herein). The web addresses for Genethon and CHLC are:

2   Genethon (http://www.genethon.fr/genethon_en.html), CHLC

3   (http://gopher.chlc.org/HomePage.html). These databases are all linked, and one of ordinary

4   skill in the art can readily access the information available from these databases.

5   The markers shown in **FIG. 6A**, from number 1 to 22 or 23 can be used to genotype

6   the CRCV pedigrees and unrelated BP-I patients described herein as well as other BP-I

7   affected individuals and pedigrees. **See FIG. 6A** (portion of a chromosome 18 map available

8   from the Whitehead Institute, web address: http://133.30.8.1:8080/=@=:www-

9   genome.wi.mit.edu. (incorporated herein by reference)). The fine mapping techniques

10   described herein in conjunction with the teachings regarding the 5 cM 18pter region can be

11   used to narrow the BP-I susceptibility region further.

12   The following markers (listed in order of occurrence from the telomere towards the

13   centromere) were used to delineate regions of BP-I susceptibility within the 5 cM 18pter

14   region: SAVA5, ca211, ca212, D18S1140, D18S59, ca231, ta201, AT201, ca225, w3442,

15   ca213, ga201, ga203, ca219, D18S1105, ca209, ca202, D18S1146, GATA (referred to in the

16   figures as 166d05) and D18S476. The markers SAVA5, D18S1140, D18S59, ta201, at201,

17   w3442, ga201, ga203, D18S1105, D18S1146, GATA and D18S476 were used in both the

18   haplotype analysis (Figure 8) and the AHR analysis (Figure 11) to delineate the BP-I

19   susceptibility locus to the 500 kb region defined by the markers SAVA5 and ga203 and the

20   300 kb region defined by D18S1140 and W3422. The other markers were used in both

21   haplotype and the AHR analyses as confirmatory evidence for the localizations. Blood

22   samples from 105 affected individuals were tested for the presence of marker haplotypes and

23   compared to marker haplotypes detected on the non-transmitted chromosome in samples

24   obtained from the parent(s) of the affected individuals when available (71 cases) or to

25   markers detected in samples obtained from a control population of students attending the

26   University of Costa Rica (52 samples). The non-transmitted chromosomes are well matched

27   as controls allowing the affected haplotype of the transmitted chromosome to be more easily

28   discerned than through comparison with data obtained from the general population that may

29   contain individuals who carry the affected haplotype but do not exhibit clinical symptoms of

30   bipolar mood disorder.

1   Figure 7 provides 18p allele frequencies for disease (aff 105) versus nontransmitted

2   (ntrans) chromosomes and samples from the control population of students (control). The

3   name of each marker used in this study is indicated on the left. The second column of

4   numbers refers to allele length in basepairs. This data provides evidence of over-

5   representation of a particular allele on disease chromosomes.

6   Figure 8 summarizes the results obtained with affected individuals. The column

7   labelled 18p refers to the patient identifier, and each patient identifier is repeated to indicate

8   results with both copies of chromosome 18. The labels "PANR" and "MANR" refer to the

9   paternal and maternal identifier, respectively, associated with the particular patient, other

10   than 0, 1 and 2, which indicate that parental samples were not available.   The allele length

11   (base pairs) is indicated under each marker for a particular patient; the length of the

12   horizontal bar in the figure reflects whether haplotypes are IBD or IBS, with IBD haplotypes

13   with common ancestors having longer bars than randomly inherited IBS haplotypes. To the

14   right of each marker, a "1" indicates that the phase is known, i.e., that it is known whether a

15   particular allele is inherited from the paternal or maternal chromosome, and a "0" indicates

16   that the phase is not known for sure. The determination of phase allows the practitioner to

17   conclude that marker alleles are linked in a haplotype on the same disease causing

18   chromosome.

19   Figure 9 provides similar data for non-transmitted chromosomes obtained from

20   parental samples. Some individuals exhibited the affected haplotype indicating that the parent

21   was homozygous; however, these regions of identity were typically much shorter than those

22   regions observed in affected individuals, indicating that they were IBS.

23   Figure 10 similarly provides data for an unscreened population of students

24   from the University of Costa Rica and their parents (52 samples in total). The data

25   demonstrate that there is a lower incidence of the affected haplotype in the general population

26   as compared with Figure 8 and that the affected haplotype is typically shorter similar to the

27   results obtained with non-transmitted chromosomes. However, the results for the general

28   population is less distinctive than that observed for non-transmitted chromosomes in allowing

29   one to map the affected haplotype.

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1   Comparison of the affected haplotype with non-transmitted chromosome markers

2   indicate that the region of maximal sharing between affected individuals occurs between

3   1140t and w3442 on chromosome 18. This region encompasses approximately 300 kb.

4   The data was analyzed further using Ancestral Haplotype Reconstruction (AHR), a

5   likelihood method for measuring LD. Data from affected individuals are examined in 2-

6   marker segments. Within each segment, the multinomial likelihood of each of the possible

7   ancestral haplotypes giving rise to the observed sample of disease haplotypes is calculated.

8   This likelihood is calculated assuming some fraction, α, of disease chromosomes are

9   associated with this 2-marker segment, and $(1-\alpha)$ are linked to this segment. These

10   haplotype likelihoods are weighted by the probability of observing that haplotype in the

11   population, and summed to create an overall likelihood for the 2-marker segment. This

12   segment likelihood is compared to the null likelihood, which assumes the disease and

13   markers are unlinked (and therefore $\alpha=0$), and a LOD score is generated. The LOD score

14   is maximized over the parameter α. Details of these calculations are presented in Appendix

15   A. The results of this analysis are shown in Figure 11. The percentages given above the

16   diagonal line demarcated by the filled boxes indicate the percentage of disease chromosomes

17   hypothesized to be true chromosomes from a common founder. For example, 17% of

18   chromosomes obtained from affected individuals have the 18S59 to W3442 region; i.e., as

19   each individual has two chromosome copies, 34% of individuals have this region. The

20   number above each percentage indicates the LOD score. The numbers given below the

21   diagonal line demarcated by the filled boxes indicate the alleles inherited from a common

22   founder, with the number prior to the dash indicating the allele of the marker identified in

23   the column heading and the number following the dash indicating the allele of the marker

24   identified in the row heading. The marker alleles are referred to as follows:

25

MARKER	#	ALLELE LENGTH
SAVA5	2	229
CA211	3	195
18S1140	2	268
18S59	4	154
18S59	6	158
TA201	2	220
TA201	3	230
CA231	2	186
CA231	4	202
AT201	1	170
AT201	2	178
CA225	1	160
CA225	3	172
W3442	1	10

Blank boxes indicate no positive evidence for linking the indicated region to the affected chromosome.

USE OF P1 CLONES TO IDENTIFY CANDIDATE cDNAS FOR SCREENING FOR MUTATIONS IN THE DNA OF BP-I PATIENTS

The P1 clones described above are used to identify candidate cDNAs. The candidate cDNAs are subsequently screened for mutations in DNA from BP-I patients. From the minimal candidate region defined by genetic mapping experiments a segment is left that is sufficiently large to contain multiple different genes.

IDENTIFICATION OF CODING SEQUENCES

Coding sequences from the surrounding DNA are identified, and these sequences are screened until a probable candidate cDNA is found. Much of the human genome will be sequenced over the next few years, in which case it may become feasible to identify coding sequences through database screening. Candidates may also be identified by scanning

1 databases consisting of partially sequenced cDNAs (Adams et al., 1991), known as expressed

2 sequence tags, or ESTs. These resources are already largely developed, and include upwards

3 of 100,000 cDNAs, the majority expressed primarily in the brain. It is not yet clear,

4 however, that the complete set of cDNAs will be mapped to specific chromosomal locations

5 in the near future, and that their data will soon be made publicly available. The database can

6 be used to identify all cDNAs that map to the minimal candidate region for BP-I. These

7 cDNAs are then used as probes to hybridize to the P1 contig, and new microsatellites are

8 isolated, which are used to genotype the "LD" sample. Maximal linkage disequilibrium in

9 the vicinity of one or two cDNAs is identified. These cDNAs are the first ones used to

10 screen patient DNA for mutations. Database screening has already been used to identify a

11 gene responsible for familial colon cancer (Papadopolous et al., 1993).

12 Coding sequences are also identified by exon amplification (Duyk et al., 1990;

13 Buckler et al., 1991). Exon amplification targets exons in genomic DNA by identifying the

14 consensus splice sequences that flank exon-intron boundaries. Briefly, exons are trapped in

15 the process of cloning genomic DNA (e.g. from P1s) into an expression vector (Zhang et al.,

16 1994). These clones are transfected into COS cells, RT-PCR is performed on total or

17 cytoplasmic RNA isolated from the COS cells using primers that are complementary to the

18 splicing vector. Exon amplification is tedious but routine; for example, the system developed

19 by Buckler et al. (1991). This method is probably preferable to another widely used

20 approach, direct selection, which involves screening cDNAs using large insert clone contigs,

21 with several steps to maximize the efficiency of hybridization and recovery of the appropriate

22 hybrid (Lovett et al., 1991). Although direct selection is more efficient than exon

23 amplification (Del Mastro et al., 1994), it may not be practical as it depends on the candidate

24 cDNA being expressed in the tissue from which the cDNA library was made; there is no

25 prior information to indicate the tissue or developmental stage in which BP-I genes would be

26 expressed.

27 Once cDNAs are identified the most plausible candidates are screened by direct

28 sequencing, SSCP or using chemical cleavage assays (Cotton et al. 1988).

29 The data are also evaluated for clues to the possible identity or mode of action of BP-

30 I mutations. For example, it is known that trinucleotide repeat expansion is associated with

1   the phenomenon of anticipation, or the tendency for a phenotype to become more severe and

2   display an earlier age of onset in the lower generations of a pedigree (Ballabio, 1993).

3   Several investigators have suggested that segregation patterns of BP-I are consistent with

4   anticipation (McInnis et al., 1993; Nylander et al., 1994). The apparent transmission of BP-

5   I, in association with the conserved 18q23 haplotype is constant with anticipation.

6   Therefore, once the candidate region is narrowed to its minimal extent, the P1 clones are

7   screened using trinucleotide repeat oligonucleotides (Hummerich et al., 1994). A PCR assay

8   is developed and patient DNAs are screened for expanded alleles.

9   Genetic and physical data help to map the bipolar mood disorder gene to the 5 cM

10 18pter region of chromosome 18. New markers from this region are tested in order to locate

11 the bipolar mood disorder gene in a region small enough to provide higher quality genetic

12 tests for bipolar mood disorder, and to specifically find the mutated gene. Narrowing down

13 the region in which the gene is located will lead to sequencing of the bipolar mood disorder

14 gene as well as cloning thereof. Further genetic analysis employing, for example, new

15 polymorphisms flanking D18S59 and D18S476 as well as the use of cosmids, yeast artificial

16 chromosome (YAC) clones, or mixtures thereof, are employed in the narrowing down

17 process. The next step in narrowing down the candidate region includes cloning of the

18 chromosomal region 18pter including proximal and distal markers in a contig formed by

19 overlapping cosmids and YACS. Subsequent subcloning in cosmids, plasmids or phages will

20 generate additional probes for more detailed mapping.

21   The next step of cloning the gene involves exon trapping, screening of cDNA

22 libraries, Northern blots or rt PCR (reverse transcriptase PCR) of samples from affected and

23 unaffected individuals, direct sequencing of exons or testing exons by SSCP (single strand

24 conformation polymorphism), RNase protection or chemical cleavage.

25   Flanking markers on both sides of the bipolar mood disorder gene combined with

26 D18S59 and D18S476 or a number of well-positioned markers that cover the chromosomal

27 region (5 cM 18pter) carrying the disease gene, can give a high probability of affected or

28 non-affected chromosomes in the range of 80-90% accuracy, depending on the

29 informativeness of the markers used and their distance from the disease gene. Using current

30 markers linked to bipolar mood disorder, and assuming closer flanking markers will be

1 identified, a genetic test for families with bipolar mood disorder will be for diagnosis in

2 conjunction with clinical evaluation, screening of risk and carrier testing in healthy siblings.

3 In the future, subsequent delineation of closely linked markers which may show strong

4 disequilibrium with the disorder, or identification of the defective gene, could allow

5 screening of the entire at-risk population to identify carriers, and provide improved

6 treatments.

7

8 ### TREATMENT OF BP-I PATIENTS USING GENOTYPE DATA

9 Using the fine mapping techniques described herein, BP-I susceptibility loci or genes

10 in the 5 cM 18pter region in particular in the region #1 between SAVA5 and ga203, are

11 identified and used to genotype patients diagnosed phenotypically with BP-I. Preferably,

12 markers in the roughly 500 kb region defined by SAVA5 and ga203, inclusive, are used.

13 More preferably, markers in either the region defined by D18S59 and w3422, inclusive, are

14 used.

15 Genotyping with the markers described herein as well as additional markers permits

16 confirmation of phenotypic BP-I diagnoses or assist with ambiguous clinical phenotypes

17 which make it difficult to distinguish between BP-I and other possible psychiatric illnesses.

18 A patient's genotype in the 5 cM 18pter region is determined and compared with previously

19 determined genotypes of other individuals previously diagnosed with BP-I. Once an

20 individual is genotyped as having a BP-I susceptibility locus in the 5 cM 18pter region, the

21 individual is treated with any of the known methods effective in treating at least certain

22 individuals affected with BP-I, such as the administration of lithium salts, carbamazepine or

23 valproic acid.

24 Studies are conducted correlating effective treatments with BP-I genotypes in the 5

25 cM 18pter region to determine the most effective treatments for particular genotypes. BP-I

26 patients can then be genotyped in the 5 cM 18pter region and the statistically most effective

27 treatment can be determined as a first course of therapy.

28 All publications and patent applications mentioned in this specification are herein

29 incorporated by reference to the same extent as if each individual publication or patent

30 application was specifically and individually indicated to be incorporated by reference.

1   The invention now being fully described, it will be apparent to one of ordinary skill

2   in the art that many changes and modifications can be made thereto without departing from

3   the spirit or scope of the appended claims.

Appendix A

Consider the original mutation to have occurred on a chromosomal segment between two markers A and B. At the time the mutation was introduced, there were n_a alleles at marker A and n_b alleles at marker B. On the chromosome containing the disease mutation both marker A and marker B carried allele X. The probability that after g generations an affected individual carrying the original disease mutation would still have allele X at markers A and B is:

$$(1-\theta_1)^g(1-\theta_2)^g + (1-\theta_1)^g(1-(1-\theta_2)^g)f(X_B) + (1-(1-\theta_1)^g)(1-\theta_2)^g f(X_A) +$$

eq (1)
$$(1-(1-\theta_1)^g)(1-(1-\theta_2)^g)f(X_A)f(X_B)$$

where θ_1 is the recombination fraction between disease and marker A, θ_2 is the recombination fraction between disease and marker B, g is the number of generations since founding (i.e. since the mutation was introduced into the population), $f(X_A)$ is the population frequency of the X-allele at marker A and $f(X_B)$ is the population frequency of the X-allele at marker B. This equation includes terms for the possibility of recombination between the markers and the disease locus, with the X-allele at the markers then being identical by state (IBS) rather than IBD. The probabilities of an affected individual with the original mutation having other haplotypes can be formulated similarly. The probability of having allele Z at marker B (where Z is any allele at marker B besides X) would be:

$$(1-\theta_1)^g(1-(1-\theta_2)^g)f(Z_B) + (1-(1-\theta_1)^g)(1-(1-\theta_2)^g)f(X_A)f(Z_B)$$

eq (2)

where $f(Z_B)$ is the frequency of allele Z at marker B in the population. The probability of having allele Z at marker A (where Z is any allele at marker B besides X) would be :

$$(1-\theta_2)^g(1-(1-\theta_1)^g)f(Z_A) + (1-(1-\theta_1)^g)(1-(1-\theta_2)^g)f(X_B)f(Z_A)$$

eq (3)

where $f(Z_A)$ is the frequency of allele Z at marker A in the population. Finally, the probability of having allele Z at both markers A and B would be:

$$(1-(1-\theta_1)^g)(1-(1-\theta_2)^g)f(Z_A)f(Z_B)$$

eq (4)

These probabilities assume (1) no interference in recombination and (2) the same marker alleles are present now as were present g generations ago, in similar frequencies. If, for example, marker A has n_a alleles and marker B has n_b alleles, then these probabilities form a $(n_a) \cdot (n_b)$ by $(n_a) \cdot (n_b)$ transition matrix, with row i containing the probabilities that founder haplotype i gave rise to each of the $(n_a) \cdot (n_b)$ different haplotypes in g generations. The rows of this transition matrix sum to 1.

37.

In simulations, the haplotype frequencies in the disease population were formulated using these transition probabilities, assuming the disease arose on a haplotype with the "1" allele at each of the two markers.

Once these transition probabilities are estimated, the likelihood of a particular founder chromosome giving rise to the observed sample of disease haplotypes in g generations is easily estimated. For example, if one assumed that the disease mutation arose on a chromosome with the X-allele at both markers, the likelihood (L_{X-X}) that this chromosome was the founder of the present-day sampled disease chromosomes is given by the multinomial:

$$L_{X-X} = \prod_{i=1}^{K} (p_{X-X,i})^{Y_i}$$

eq (5)

where i indexes the K potential haplotypes for the two markers ($K = (n_a)(n_b)$), $p_{X-X,i}$ is the probability that the ancestral disease chromosome with the X-allele at both markers gave rise to a haplotype of type i in g generations (taken from the transition matrix), and Y_i is the observed number of haplotype i in the sample ($\Sigma_i(Y_i)$=the number of chromosomes in the sample to be analyzed). The likelihood in eq (5) assumes that all affected individuals are independent. While, after many generations of separation from a common ancestor one might consider these

38.

individuals to be independent, they are in fact related through a complex and un-known pedigree. The simplification of considering individuals as independent makes the likelihood much more tractable to compute.

The K likelihoods are then summed, and weighted by the probability of observing that particular haplotype in the population to produce an overall likelihood for the 2-marker segment:

eq (6)
$$L = \sum_{i=1}^{K} f_i L_i$$

where f_i is the frequency of haplotype i in the population. This overall likelihood calculation parallels the approach taken by Terwilliger (1995, eq (2)). The haplotype frequencies are estimated from the sample of normal chromosomes. In the event that the haplotype resulting in the largest contribution to the overall likelihood in eq (6) is not observed in the normal sample, the upper 95% confi-dence interval for this frequency is used, and the remaining haplotype frequencies rescaled accordingly.

This overall likelihood is compared to the null likelihood, which is generated in exactly the same manner, except that it is assumed the markers were unlinked to

the disease locus ($\theta_1=\theta_2=0.5$ in, for example, eqs (1-4)). The $\log_{10}$ of this likeli-hood ratio is a LOD score. One might consider to use in the null likelihood transition probabilities calculated under the assumption of linkage equilibrium. Under this null the cells of the transition matrix are computed by multiplication of allele frequencies, assuming independence of marker loci. These two forms of the null likelihood are equivalent in value for g of approximately 20 or greater, and for $g<20$ the values are nearly equivalent.

Because θ_1 and θ_2 are obviously unknown, the putative disease locus is set to be in the middle of the segment and therefore θ_1 and θ_2 are one-half the genetic distance (converted to recombination fraction by the Haldane mapping function, (Ott 1991)) between the two marker loci forming the segment. In fact, one could estimate θ_1 and θ_2, or their ratio, and the method could easily be modified to do so, however for our purposes finding a linked segment is suitable.

This basic procedure has been modified to deal with heterogeneity in the sample of disease chromosomes. Not all chromosomes in the disease sample may be true disease chromosomes from a common founder. Individuals heterozygous for the disease mutation will add one chromosome to the disease sample that will not be a true disease chromosome. Additionally, affected individuals not linked to the

40.

particular chromosomal segment being analyzed (either because they are pheno-copies or because of locus heterogeneity) will contribute two chromosomes to the affected sample that do not harbor this disease locus. When the null hypothesis of no linkage is not true, some fraction, α, of the chromosomes in the disease sample will associated with this chromosomal segment, and $(1-\alpha)$ will not be associated. We decided to examine α in steps of 0.1, from 1.0 to 0.0, and for each step in α produce a new transition matrix under the alternative hypothesis and calculate a LOD score. If we call the transition matrix calculated under the alternative hypothesis (where the disease locus is hypothesized to be in the middle of the 2-marker segment) T_a and call the transition matrix calculated under the null hypothesis (where the disease locus is unlinked to the 2-marker segment) T_n, then a new transition matrix for the alternative hypothesis is calculated as:

$$T^{*}_{a} = \alpha T_{a} + (1-\alpha)T_{n}$$

eq (7)

The transition matrix under the null uses $\alpha=0$. The LOD score is then maximized over the one parameter α.

1   <u>WHAT IS CLAIMED IS</u>:

2

3   1.   A method of detecting the presence of a bipolar mood disorder susceptibility locus in

4   an individual comprising:

5   analyzing a sample of DNA from said individual for the presence of a DNA

6   polymorphism on the short arm of chromosome 18 between SAVA5 and ga203, wherein said

7   DNA polymorphism is associated with a form of bipolar mood disorder.

8

9   2.   The method of claim 1, wherein said DNA polymorphism is located on the short arm

10 of chromosome 18 between D18S1140 and ga203, inclusive.

11

12 3.   The method of claim 1, wherein said DNA polymorphism is located on the short arm

13 of chromosome 18 between SAVA5 and W3422, inclusive.

14

15 4.   The method of claim 1, wherein said DNA polymorphism is located on the short arm

16 of chromosome 18 between D18S1140 and W3422, inclusive.

17 ·

18 5.   The method of claim 1, wherein said DNA polymorphism is located on the short arm

19 of chromosome 18 between D18S1140 and at201, inclusive.

20

21 6.   The method of claim 1, wherein said DNA polymorphism is located on the short arm

22 of chromosome 18 between D18S1140 and ta201, inclusive.

23

24 7.   The method of claim 1, wherein said DNA polymorphism is located on the short arm

25 of chromosome 18 between D18S59 and ta201, inclusive.

26

1   8.   The method of claim 1, wherein said analyzing further comprises:

2   a. obtaining DNA samples from family members of said individual,

3   b. analyzing said DNA samples from family members for the presence of said DNA

4   polymorphism, and

5   c.   correlating the presence or absence of the DNA polymorphism with a

6   phenotypic diagnosis of bipolar mood disorder for said individual and for said family

7   members.

8

9   9.   A method for detecting the presence of a DNA polymorphism linked to a gene

10   associated with bipolar mood disorder in an individual comprising:

11   a.   typing blood relatives of said individual for a DNA polymorphism located

12   within a 500kb region of chromosome 18, wherein said region is located between SAVA5

13   and ga203, inclusive,

14   b.   analyzing a DNA sample from said individual for the presence of said DNA

15   polymorphism.

16

17   10.   A method of genetically diagnosing bipolar mood disorder in an individual

18   comprising:

19   a.   obtaining a DNA sample from said individual,

20   b.   analyzing said DNA sample for the presence of a DNA polymorphism

21   associated with bipolar mood disorder, wherein said DNA polymorphism is located within a

22   500 kb region of chromosome 18, wherein said region is located between SAVA5 and ga203,

23   inclusive.

24

25   11.   A method of confirming a phenotypic diagnosis of bipolar mood disorder in an

26   individual comprising:

27   a.   obtaining a DNA sample from said individual,

28   b.   analyzing said DNA sample for the presence of a DNA polymorphism

29   associated with bipolar mood disorder, wherein said DNA polymorphism is located within a

1 500 kb region of chromosome 18, wherein said region is located between SAVA5 and ga203,

2 inclusive.

3

4 12. The method of claim 10, wherein said individual has Spanish or Amerindian ancestry.

5

6 13. A method of classifying subtypes of bipolar mood disorder comprising:

7 a. identifying one or more DNA polymorphisms located within a 500 kb region

8 of chromosome 18, wherein said region is located between SAVA5 and ga203, inclusive; and

9

10 b. analyzing DNA samples from individuals phenotypically diagnosed with

11 bipolar mood disorder for the presence or absence of one of more of said DNA

12 polymorphisms.

13

14 14. A method of treating an individual diagnosed with bipolar mood disorder comprising:

15 a. identifying one or more DNA polymorphisms located within a 500 kb region

16 of chromosome 18, wherein said region is located between SAVA5 and ga203, inclusive; and

17

18 b. analyzing DNA samples from individuals phenotypically diagnosed with

19 bipolar mood disorder for the presence or absence of one of more of said DNA

20 polymorphisms, and

21 c. selecting a treatment plan that is most effective for individuals having a

22 particular genotype within said 500 kb region of chromosome 18.

23

24 15. An isolated polynucleotide capable of selectively hybridizing with a DNA sample

25 from an individual phenotypically diagnosed with severe bipolar mood disorder, wherein said

26 polynucleotide does not selectively hybridize with a DNA sample from an individual not

27 affected by severe bipolar mood disorder, wherein said isolated polynucleotide selectively

28 hybridizes with a complementary polynucleotide within a 500 kb region of chromosome 18,

29 wherein said region is located between SAVA5 and ga203, inclusive.

30

1 16. The isolated polynucleotide of claim 15, wherein said complementary polynucleotide

2 is within a 500 kb region of chromosome 18, between SAVA5 and ga203, inclusive.

3

1
2
3
4
5 METHODS FOR TREATING BIPOLAR MOOD DISORDER

6 ASSOCIATED WITH MARKERS ON CHROMOSOME 18p

7

8

9 ABSTRACT OF THE DISCLOSURE

10 The present invention is directed to methods of detecting the presence of a bipolar

11 mood disorder susceptibility locus in an individual, comprising analyzing a sample of DNA

12 for the presence of a DNA polymorphism on the short arm of chromosome 18 between the

13 telomere and D18S481, wherein the DNA polymorphism is associated with a form of bipolar

14 mood disorder. The invention for the first time provides strong evidence of a susceptibility

15 gene for bipolar mood disorder that is located in the terminal 5 cM region of the short arm

16 of chromosome 18. The disclosure describes the use of linkage analysis and genetic markers

17 in this 5 cM region to fine map the region and the use of genetic markers to genetically

18 diagnose (genotype) bipolar mood disorder in individuals, to confirm phenotypic diagnoses of

19 bipolar mood disorder, to determine appropriate treatments for patients with particular

20 genotypic subtypes. Isolated polynucleotides useful for genetic linkage analysis of BP-I and

21 methods for obtaining such isolated polynucleotides are also described.

22

FIG 1

Figure 2
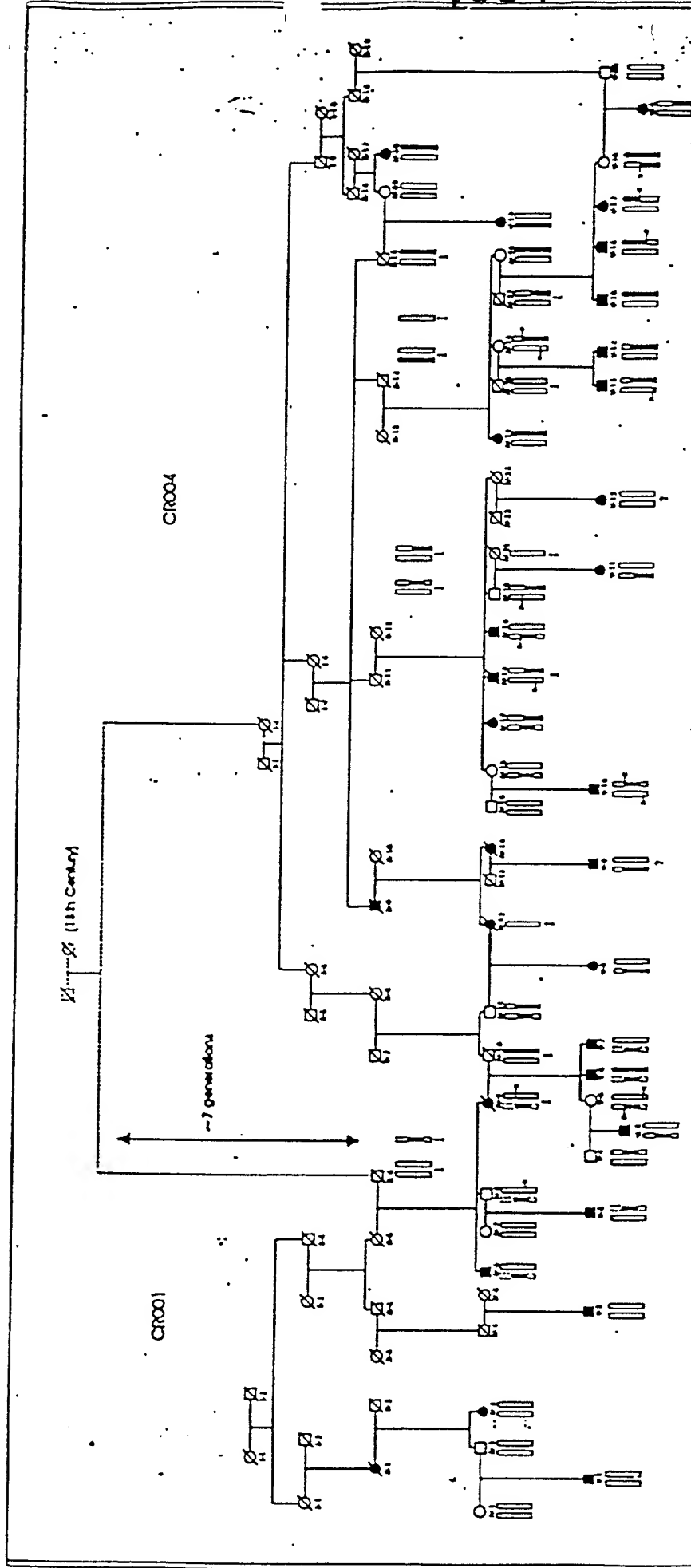
Table 1. Lod scores for markers exceeding the arbitrary coverage thresholds.

Marker Name	distance from pter	Family CR001		Family CR004		Combined	
		Z_{max} ≥ 0.8	Theta	Z_{max} ≥ 1.2	Theta	Z_{max} ≥ 1.6	Theta
D1S456	224.6	1.32	0.0	0.0	0.50	0.0	0.50
D2S130	230.1	0.89	0.0	0.12	0.35	0.36	0.26
D3S1285	91.0	0.00	0.50	2.59	0.00	1.15	0.16
D4S171	207.9	1.07	0.07	0.01	0.05	0.22	0.29
D5S427	69.6	1.39	0.0	0.0	0.50	0.7	0.18
D7S510	60.5	0.04	0.40	2.04	0.0	0.82	0.17
D11S929	36.3	0.80	0.11	0.03	0.42	0.43	0.24
D11S1392	38.6	0.86	0.07	0.90	0.23	1.58	0.19
D11S1312	42.0	0.47	0.13	1.77	0.0	1.95	0.05
D13S175	7.4	0.83	0.0	0.0	0.50	0.24	0.15
D15S126	45.5	1.09	0.0	0.0	0.48	0.06	0.40
D16S521	4.6	1.46	0.0	0.41	0.26	1.18	0.17
D16S515	94.8	0.93	0.09	0.01	0.46	0.39	0.25
D16S486	133.6	0.27	0.19	1.29	0.20	1.60	0.20
D17S849	0.60	0.0	0.50	1.22	0.07	0.32	0.14
D18S59	1.1	1.43	0.0	0.0	0.50	0.02	0.46
D18S1105	2.8	0.97	0.0	0.01	0.47	0.01	0.46
D18S71	43.8	0.96	0.0	0.0	0.50	0.0	0.50
D18S64	84.0	0.33	0.11	1.34	0.15	1.67	0.13
D18S55	95.5	0.0	0.50	2.09	0.13	1.51	0.18
D18S61	103.8	0.0	0.50	2.26	0.12	1.94	0.16
D18S488	105.6	0.0	0.50	1.26	0.14	1.02	0.19
D18S1161	113.0	0.0	0.50	1.79	0.16	1.76	0.17

Markers for which lod scores exceeded the arbitrary thresholds used for genome coverage calculations (in bold). Z_{max} is the maximum likelihood estimate of the lod score at the corresponding value of the recombination fraction (theta).

4
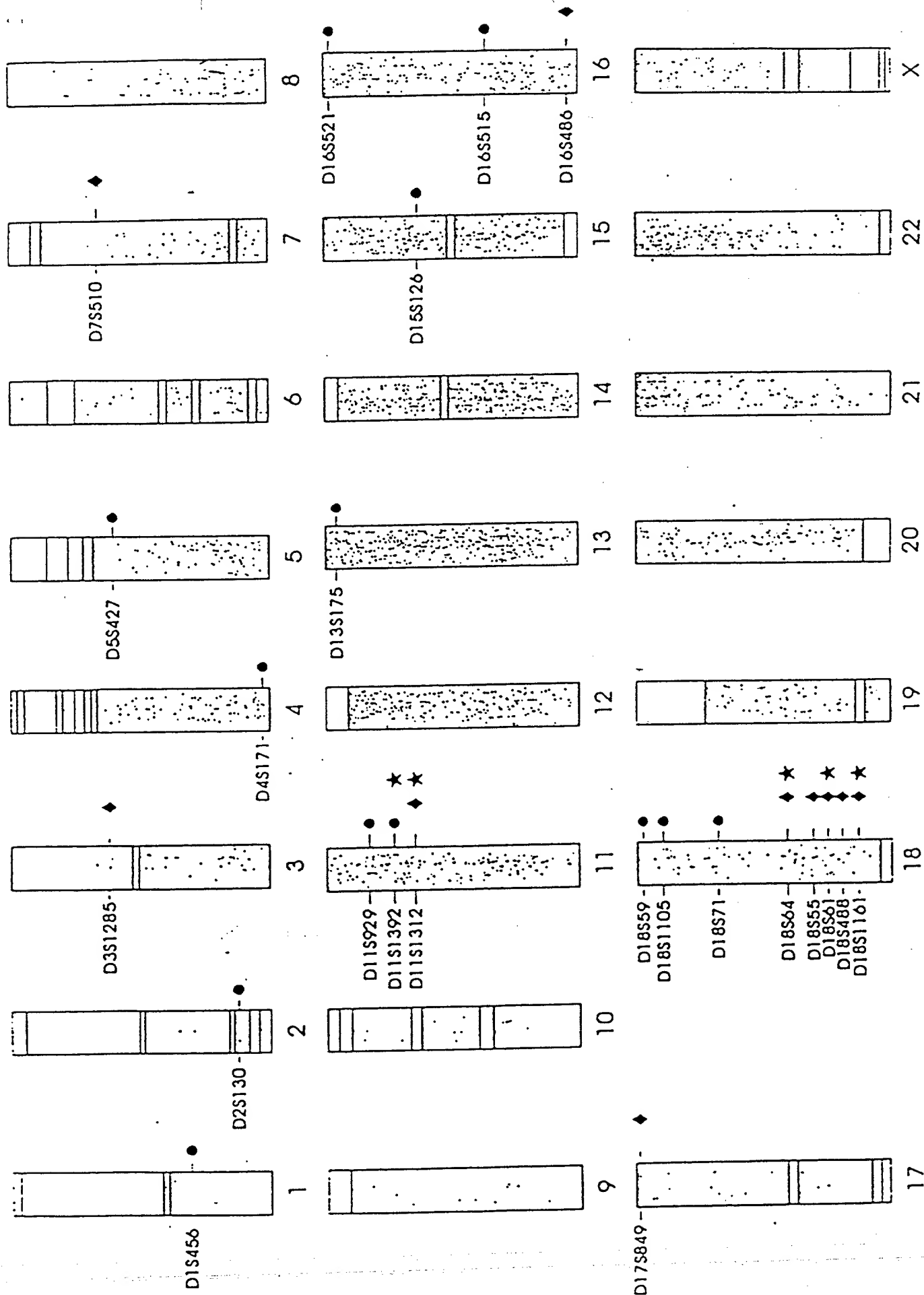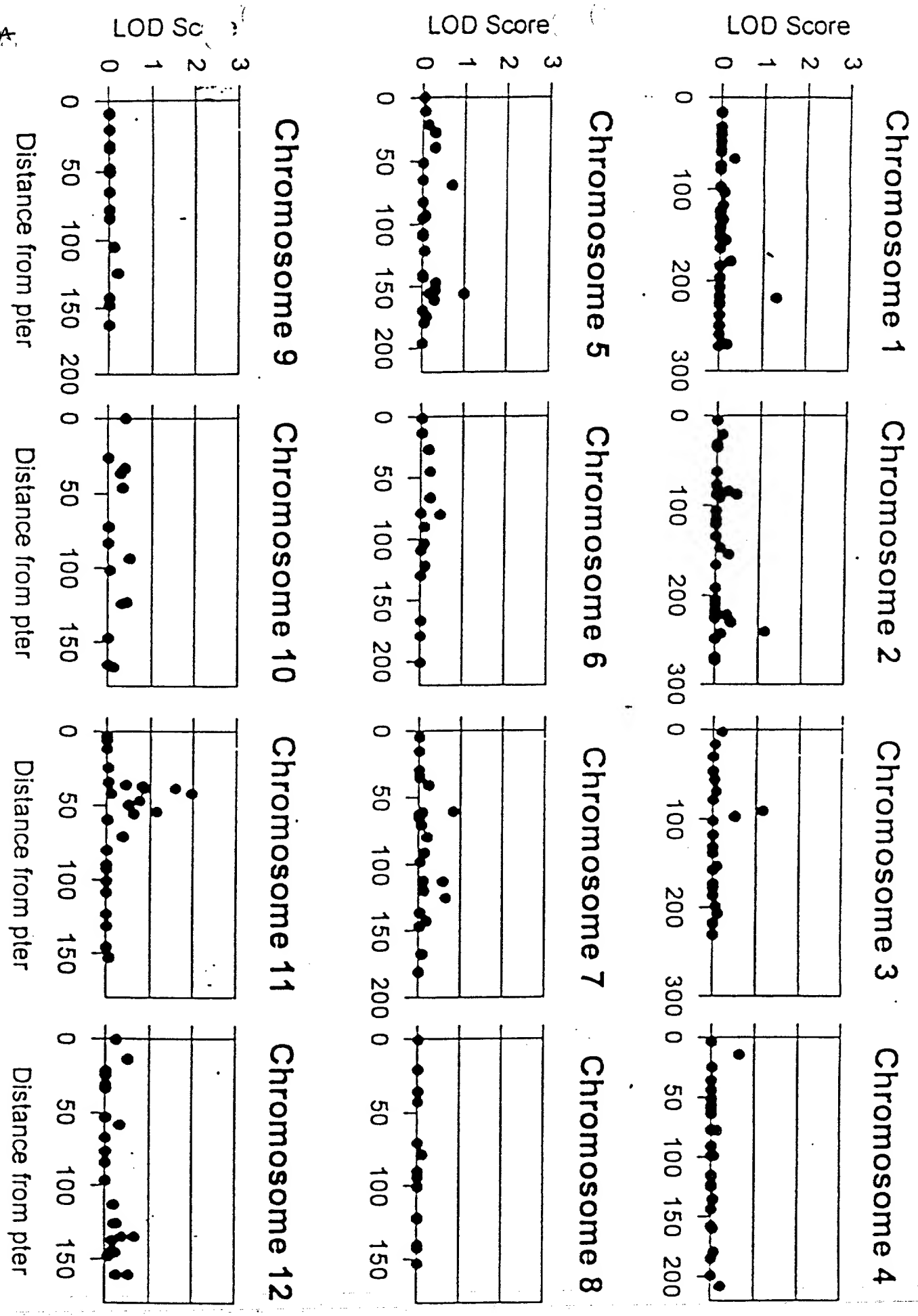
FIGURE 3
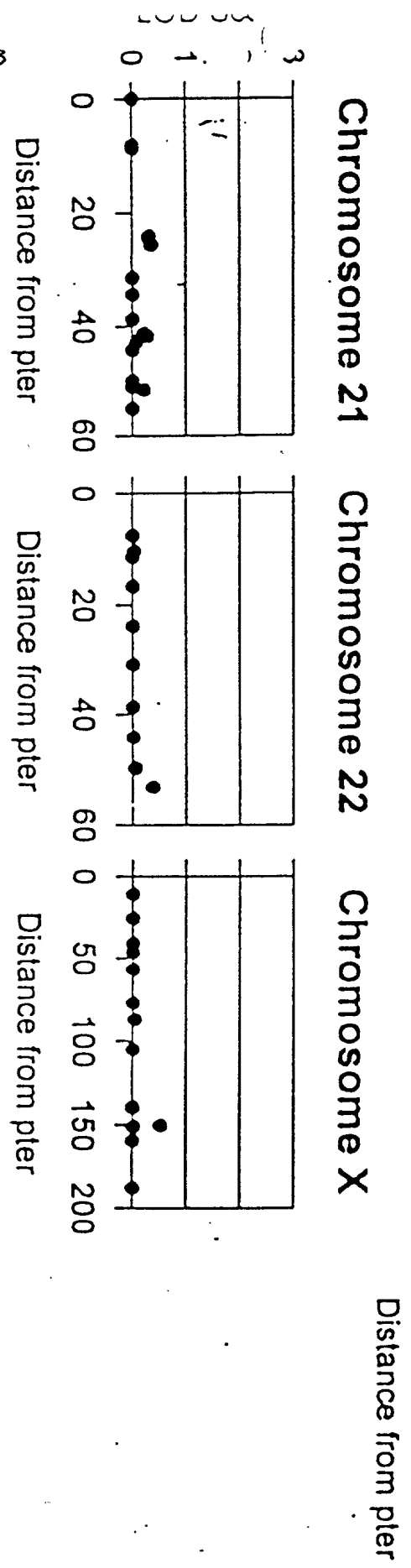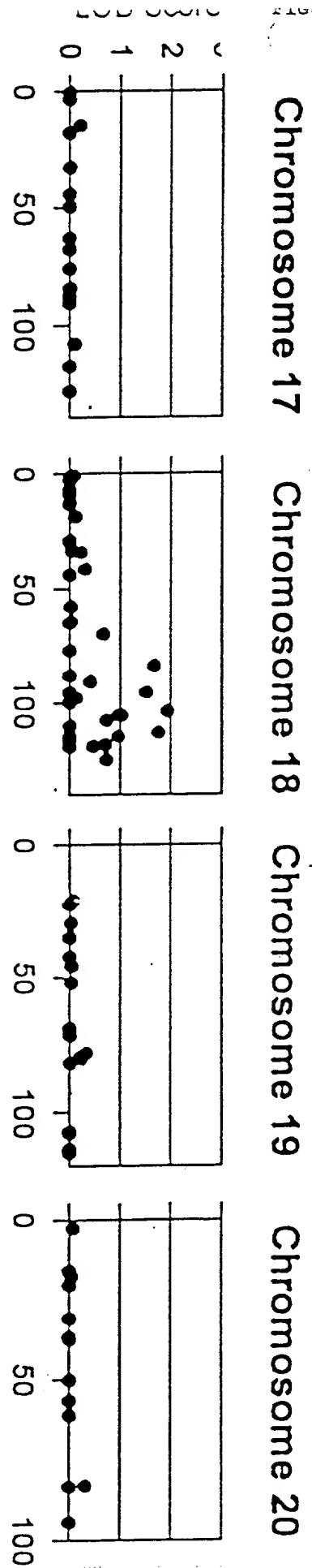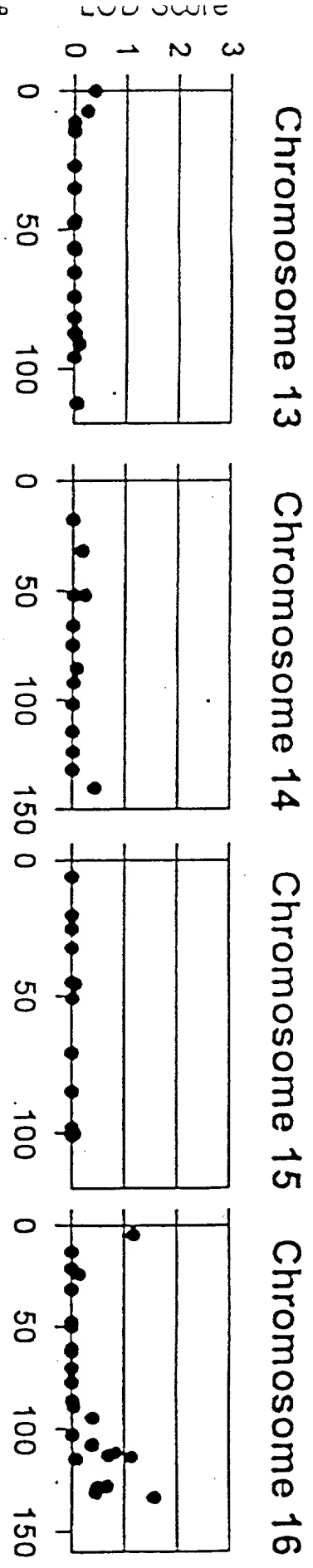
Figure 4A

Figure 4.B

FIGURE 5

Chr18: Contigs Anchored on Integrated Map

Be patient... This is a large image!

Chromosome Chr18
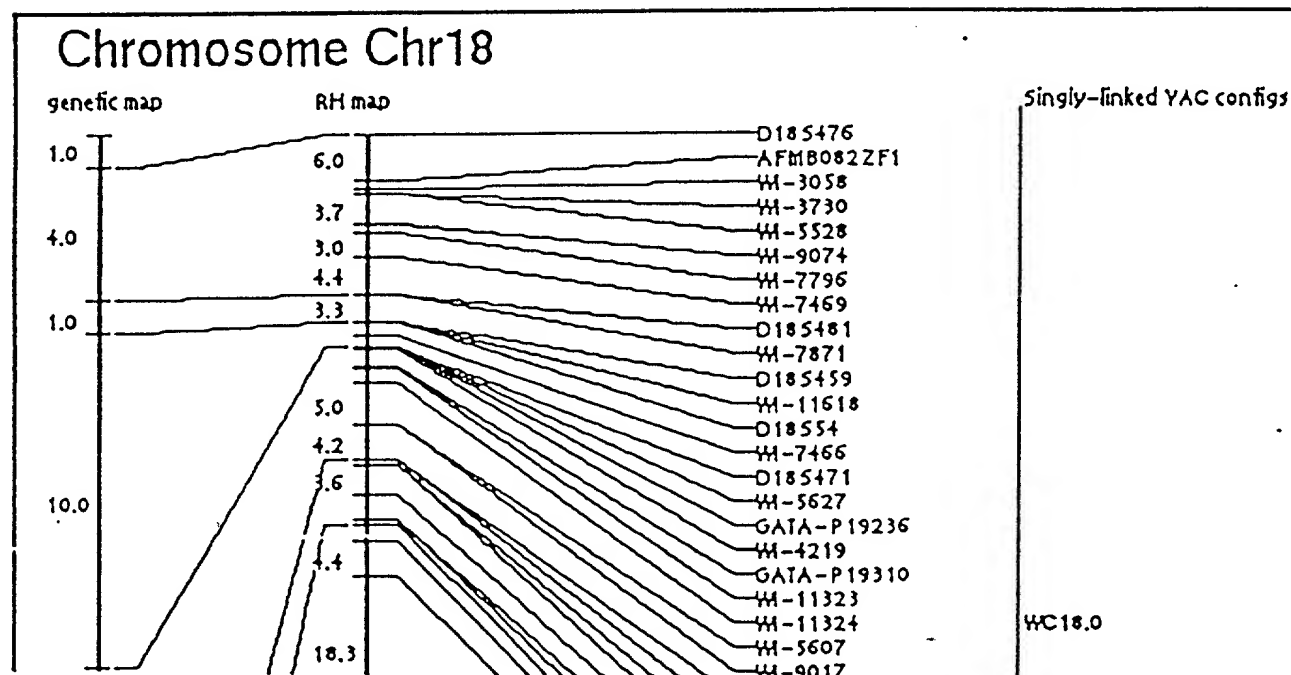
You can click on the name of an STS or a contig in order to retrieve information about it. Download this map as a PICT file (Macintosh) or a GIF (everybody else)

NOTES

1. This is a composite map in which the genetic linkage map from Généthon, and the radiation hybrid map from the Whitehead Institute/MIT Center are used to anchor YAC/STS contigs. We only show the subset of genetic- and radiation-hybrid mapped STSs for which positive YACs are present. For the genetic map, please refer to the linkage maps published in *Nature Genetics 7(2):246-339 (1994)* for the complete genetic maps.
2. The apparent size of a contig on this map does not always correlate with the number of its members. Some apparent "large" contigs are artifically expanded because of contradictions between the radiation hybrid map position of one or more markers on the genetic map, and adjacencies computed from YAC linkage. Contigs that appear to overlap may represent places where missing YAC data prevents the contigs from merging, or, in some cases, contradictions between the order derived from the radiation hybrid map and the order derived from the STS content map.
3. The large central gap that appears on many of the radiation hybrid maps corresponds to the centromere.
4. Markers derived from expressed sequence tags (ESTs) or other expressed sequences are colored red.

This STS is part of singly-linked contig <u>WC18.0</u>:

	STS	Chrom	Map Position Genetic	RH	Contig Single	Double
1	<u>WI-9527</u>	<u>Chr18</u>	-	-	<u>WC18.0</u>	<u>WC-1465</u>
2	<u>CHLC.GGAT2G04</u>	<u>Chr18</u>	-	-	<u>WC18.0</u>	<u>WC-1465</u>
3	<u>CHLC.GGAT2G04.1217</u>	<u>Chr18</u>	-	-	<u>WC18.0</u>	<u>WC-1465</u>
4	<u>D18S59</u>	<u>Chr18</u>	<u>0 cM</u>	-	<u>WC18.0</u>	<u>WC-1465</u>
5	<u>D18S1140</u>	<u>Chr18</u>	<u>0 cM</u>	-	<u>WC18.0</u>	<u>WC-1465</u>
6	<u>WI-7796</u>	<u>Chr18</u>	-	<u>15 cR</u>	<u>WC18.0</u>	-
7	<u>WI-9074</u>	<u>Chr18</u>	-	<u>12 cR</u>	<u>WC18.0</u>	<u>WC-1465</u>
8	<u>WI-5528</u>	<u>Chr18</u>	-	<u>7 cR</u>	<u>WC18.0</u>	-
9	<u>D18S476</u>	<u>Chr18</u>	<u>1 cM</u>	<u>0 cR</u>	<u>WC18.0</u>	-
10	<u>WI-7226</u>	<u>Chr18</u>	-	-	<u>WC18.0</u>	<u>WC-909</u>
11	<u>AFMB324ZE5</u>	<u>Chr18</u>	-	-	<u>WC18.0</u>	<u>WC-909</u>
12	<u>AFMB082ZF1</u>	<u>Chr18</u>	-	<u>5 cR</u>	<u>WC18.0</u>	<u>WC-909</u>
13	<u>D18S1146</u>	<u>Chr18</u>	<u>1 cM</u>	-	<u>WC18.0</u>	<u>WC-909</u>
14	<u>WI-3058</u>	<u>Chr18</u>	-	<u>5 cR</u>	<u>WC18.0</u>	<u>WC-909</u>
15	<u>D18S1105</u>	<u>Chr18</u>	<u>1 cM</u>	-	<u>WC18.0</u>	<u>WC-909</u>
16	<u>WI-3730</u>	<u>Chr18</u>	-	<u>5 cR</u>	<u>WC18.0</u>	<u>WC-1576</u>
17	<u>AFM077YD11</u>	<u>Chr18</u>	-	-	<u>WC18.0</u>	<u>WC-1576</u>
18	<u>D18S1098</u>	<u>Chr18</u>	<u>4 cM</u>	-	<u>WC18.0</u>	<u>WC-1576</u>
19	<u>WI-7469</u>	<u>Chr18</u>	-	<u>16 cR</u>	<u>WC18.0</u>	<u>WC-1576</u>
20	<u>WI-7871</u>	<u>Chr18</u>	-	<u>22 cR</u>	<u>WC18.0</u>	<u>WC-1576</u>
21	<u>D18S481</u>	<u>Chr18</u>	<u>5 cM</u>	<u>21 cR</u>	<u>WC18.0</u>	<u>WC-1576</u>
22	<u>WI-4747</u>	<u>Chr18</u>	-	-	<u>WC18.0</u>	<u>WC-1576</u>
23	<u>D18S1154</u>	<u>Chr18</u>	<u>6 cM</u>	-	<u>WC18.0</u>	<u>WC-1576</u>
24	<u>CHLC.ATA14B09</u>	<u>Chr18</u>	-	-	<u>WC18.0</u>	<u>WC-1576</u>
25	<u>WI-7466</u>	<u>Chr18</u>	-	<u>27 cR</u>	<u>WC18.0</u>	<u>WC-1576</u>
26	<u>D18S54</u>	<u>Chr18</u>	<u>6 cM</u>	<u>25 cR</u>	<u>WC18.0</u>	<u>WC-1576</u>
27	<u>D18S63</u>	<u>Chr18</u>	<u>6 cM</u>	-	<u>WC18.0</u>	<u>WC-1576</u>
28	<u>D18S459</u>	<u>Chr18</u>	<u>6 cM</u>	<u>25 cR</u>	<u>WC18.0</u>	<u>WC-1576</u>
29	<u>WI-6014</u>	<u>Chr18</u>	-	-	<u>WC18.0</u>	<u>WC-1576</u>
30	<u>WI-4219</u>	<u>Chr18</u>	-	<u>28 cR</u>	<u>WC18.0</u>	<u>WC-143</u>
31	<u>AFM238YG3</u>	<u>Chr18</u>	-	-	<u>WC18.0</u>	<u>WC-143</u>
32	<u>D18S471</u>	<u>Chr18</u>	<u>17 cM</u>	<u>28 cR</u>	<u>WC18.0</u>	<u>WC-143</u>
33	<u>D18S458</u>	<u>Chr18</u>	<u>17 cM</u>	-	<u>WC18.0</u>	<u>WC-143</u>

34	D18S452	Chr18	17 cM	-	WC18.0 WC-143
35	D18S62	Chr18	17 cM	-	WC18.0 WC-143
36	WI-5627	Chr18	-	28 cR	WC18.0 WC-143
37	CHLC.GATA82D03	Chr18	-	28 cR	WC18.0 WC-143
38	FB25F12	Chr18	-	-	WC18.0 WC-143
39	CHLC.GATA51H07	Chr18	-	-	WC18.0 WC-143
40	CHLC.GATA88A12	Chr18	-	30 cR	WC18.0 WC-143
41	WI-9619	Chr18	-	-	WC18.0 WC-143
42	AFMB346YA9	Chr18	-	-	WC18.0 WC-143
43	AFM323TC9	Chr18	-	-	WC18.0 WC-862
44	WI-5607	Chr18	-	36 cR	WC18.0 WC-862
45	WI-9017	Chr18	-	36 cR	WC18.0 WC-862
46	AFM077YF7	Chr18	-	-	WC18.0 WC-934
47	WI-8546	Chr18	-	-	WC18.0 WC-934
48	CHLC.GGAA16G02	Chr18	-	-	WC18.0 WC-934
49	D18S464	Chr18	32 cM	46 cR	WC18.0 WC-934
50	NIB1802	Chr18	-	56 cR	WC18.0 WC-934
51	D18S1153	Chr18	34 cM	-	WC18.0 WC-934
52	D18S1150	Chr18	36 cM	-	WC18.0 WC-934
53	WI-4589	Chr18	-	58 cR	WC18.0 WC-934
54	WI-4319	Chr18	-	62 cR	WC18.0 WC-934
55	D18S1158	Chr18	38 cM	-	WC18.0 WC-934
56	D18S1116	Chr18	40 cM	-	WC18.0 WC-377
57	CHLC.GATA11A06.668	Chr18	-	48 cR	WC18.0 WC-377
58	CHLC.GATA11A06	Chr18	-	54 cR	WC18.0 WC-377
59	D18S53	Chr18	41 cM	-	WC18.0 WC-377
60	WI-9134	Chr18	-	52 cR	WC18.0 WC-377
61	IB1114	Chr18	-	-	WC18.0 WC-377
62	D18S482	Chr18	41 cM	56 cR	WC18.0 WC-377
63	WI-2382	Chr18	-	-	WC18.0 WC-377
64	WI-6819	Chr18	-	-	WC18.0 WC-377
65	D18S71	Chr18	43 cM	84 cR	WC18.0 WC-377
66	AFMA058YG5	Chr18	-	80 cR	WC18.0 WC-377
67	WI-5506	Chr18	-	90 cR	WC18.0 WC-377
68	D18S453	Chr18	43 cM	93 cR	WC18.0 WC-738
69	D18S73	Chr18	43 cM	-	WC18.0 WC-377
70	STSG-10174	Chr18	-	-	WC18.0 WC-377

71	CHLC.GCT5D07	Chr18	-	101 cR	WC18.0	WC-377
72	WI-10768	Chr18	-	-	WC18.0	WC-1182
73	D18S1149	Chr18	49 cM	-	WC18.0	WC-1182
74	WI-1869	Chr18	-	-	WC18.0	WC-1182
75	D18S1104	Chr18	49 cM	-	WC18.0	WC-1182
76	AFMA205YH5	Chr18	-	-	WC18.0	WC-1182
77	AFMB340VE5	Chr18	-	-	WC18.0	WC-1182
78	CHLC.GATA41G05	Chr18	-	185 cR	WC18.0	WC-1182
79	AFMB319WF9	Chr18	-	-	WC18.0	WC-1182
80	D18S44	Chr18	-	-	WC18.0	WC-1182

Details on contig assembly.

Figure 7

18p allele frequencies

MARKERNAME	aff 105	ntrans	control
D18SAVA5			
225	0.04	0.02	
227	**0.29**	**0.24**	
229	**0.22**	**0.15**	
231	0.04	0.08	
233	0.14	0.23	
235	0.25	0.22	
237	0.02	0.03	
239	0.00	0.00	
D18SCA211			
183	0.02	0.04	0.01
189	0.00	0.01	0.01
191	0.01	0.00	0.03
193	0.24	0.17	0.33
195	0.21	0.19	0.18
197	0.06	0.11	0.03
199	0.06	0.04	0.01
201	0.10	0.14	0.10
203	0.02	0.04	0.06
205	0.16	0.18	0.14
207	0.09	0.04	0.06
209	0.02	0.02	0.02
211	0.01	0.00	0.00
215	0.00	0.00	0.00
217	0.00	0.00	0.01
D18SCA212			
200	**0.40**	**0.40**	**0.39**
202	0.31	0.32	0.29
204	0.05	0.05	0.03
206	0.04	0.06	0.10
214	0.01	0.00	0.00
216	0.14	0.12	0.15

MARKERNAME		aff 105	ntrans	control		
	218	0.04	0.00	0.04		
D18S1140	256	0.06	0.07	0.06		
	268	0.77	0.72	0.73		
	270	0.02	0.00	0.06		
	272	0.03	0.03	0.03		
	274	0.00	0.00	0.00		
	276	0.03	0.06	0.02		
	278	0.02	0.06	0.05		
	280	0.04	0.06	0.02		
	282	0.01	0.00	0.02		
MARKERNAME		aff 105	ntrans	control		
D18S59	148	0.16	0.26	0.21		
	150	0.07	0.09	0.14		
	152	0.02	0.06	0.01		
	154	0.36	0.19	0.28	0.17	0.08
	156	0.04	0.04	0.08		
	158	0.22	0.21	0.13		
	160	0.04	0.08	0.05		
	162	0.05	0.06	0.05		
	164	0.02	0.01	0.02		
	168	0.00	0.00	0.01		
D18STA201	214	0.02	0.00	0.00		
	220	0.09	0.09	0.04		
	222	0.01	0.00	0.01		
	228	0.01	0.01	0.00		
	230	0.25	0.22	0.16	0.03	0.09

18p allele frequencies

MARKERNAME		aff 105	ntrans	control
	232	0.07	0.04	0.07
	234	0.02	0.00	0.00
	236	0.01	0.00	0.00
	238	0.01	0.00	0.00
	242	0.09	0.09	0.04
	244	0.13	0.13	0.19
	246	0.09	0.09	0.11
	248	0.06	0.11	0.10
	250	0.07	0.07	0.06
	252	0.07	0.10	0.12
	254	0.02	0.03	0.03
	256	0.01	0.01	0.03
	258	0.01	0.01	0.01
	260	0.01	0.09	0.02
	262	0.01	0.00	0.00
D18SCA231	182	0.00	0.00	0
	184	0.20	0.23	0.26
	186	0.70	0.66	0.68
	188	0.00	0.01	0.01
	190	0.02	0.00	0.02
	192	0.00	0.00	0.01
	194	0.02	0.02	0
	196	0.00	0.00	0
	198	0.02	0.01	0
	200	0.01	0.01	0.01
	202	0.02	0.03	0.01

MARKERNAME	aff 105	ntrans	control

18p allele frequencies

MARKERNAME		aff 105	ntrans	control		
D18SAT201	170	0.53	0.55	0.52		
	174	0.00	0.01	0.01		
	178	0.37	0.36	0.36		
	182	0.01	0.00	0.00		
	186	0.07	0.06	0.07		
	190	0.01	0.00	0.00		
	194	0.01	0.01	0.03		
D18SCA225	160	0.16	0.20	0.21		
	168	0.02	0.04	0.00		
	170	0.00	0.00	0.01		
	172	**0.47**	**0.38**	**0.42**	0.09	0.04
	174	0.22	0.24	0.26		
	176	0.04	0.04	0.05		
	178	0.04	0.04	0.02		
	180	0.02	0.01	0.01		
	184	0.03	0.00	0.02		
D18SW3442	10	**0.42**	**0.28**	**0.36**	**0.14**	**0.06**
	12	0.01	0.01	0.01		
	14	0.07	0.11	0.11		
	16	0.12	0.17	0.12		
	18	0.18	0.15	0.14		
	20	0.05	0.09	0.09		
	22	0.08	0.10	0.11		
	24	0.05	0.08	0.03		
	26	0.00	0.00	0.02		
	38	0.00	0.00	0.00		
D18SCA213	112	0.12	0.17	0.07		
	120	0.00	0.05	0.01		
	122	0.03	0.03	0.04		
	124	0.44	0.37	0.46		

FIGURE 1. 18p allele frequencies

MARKERNAME		aff 105	ntrans	control
	126	0.30	0.24	0.35
	128	0.08	0.11	0.06
	130	0.00	0.00	0.00
	132	0.03	0.02	0.01
D18SGAT201	142	0.04	0.06	0.02
	146	0.08	0.08	0.06
	150	**0.61**	**0.62**	**0.69**
	154	0.15	0.15	0.12
	158	0.11	0.07	0.10
	162	0.02	0.02	0.00
D18SGAT203	188	0.42	0.37	0.38
	192	0.12	0.14	0.17
	196	0.01	0.04	0.01
	200	0.02	0.04	0.01
	204	0.06	0.02	0.04
	208	0.19	0.21	0.20
	212	0.11	0.11	0.11
	216	0.09	0.07	0.08
D18SCA219	221	0.00		0.01
	223	0.00		0.00
	225	0.00		0.00
	233	0.00		0.00
	235	0.22		0.21
	239	0.02		0.01
	241	0.54		0.63
	243	0.07		0.07
	245	0.13		0.06
MARKERNAME		aff 105	ntrans	control

MARKERNAME		aff 105	ntrans	control
D18S1105	101	0.16	0.11	
	103	0.12	0.08	
	105	0.03	0.02	
	81	0.02	0.01	
	83	0.01	0.02	
	85	0.51	0.54	
	87	0.01	0.06	
	91	0.00	0.00	
	95	0.01	0.04	
	97	0.04	0.04	
	99	0.08	0.06	
D18SCA209	173	0.57	0.53	0.69
	175	0.02	0.03	0.04
	177	**0.20**	0.18	**0.09**
	179	0.01	0.03	0.00
	181	0.19	0.24	0.18
	187	0.00	0.00	0.00
D18SCA202	182	0.16	0.14	
	184	0.02	0.00	
	186	0.01	0.01	
	190	0.09	0.02	
	192	0.10	0.16	
	194	0.10	0.09	
	196	0.37	0.35	
	198	0.09	0.10	
	200	0.05	0.08	
	202	0.00	0.03	
	208	0.00	0.00	
D18S1146	270	0.32	0.35	
	272	0.07	0.10	
	274	0.60	0.51	

18p allele frequencies

MARKERNAME		aff 105	ntrans	control	
	276	0.02	0.04		
D18S166D05	300	0.17	0.21	0.19	
	304	0.16	0.12	0.14	
	308	0.18	0.18	0.13	
	312	**0.35**	**0.26**	**0.36**	**
	316	0.08	0.18	0.11	
	320	0.04	0.04	0.03	
	324	0.01	0.01	0.02	
D18S476	261	0.00	0.01	0.01	
	263	0.01	0.04	0.04	
	265	0.05	0.12	0.04	
	267	0.20	0.26	0.23	
	269	0.08	0.09	0.04	
	271	**0.56**	**0.38**	**0.54**	***
	273	0.04	0.08	0.07	
	275	0.04	0.03	0.03	

Figure 8

Affected haplotypes

18p	PAN	MAN	ca212	1140	59	ca231	ta201	at201	PDca225	w3442	ca213	ga201	ga203
200	279	280	218	1 268	1 158	1 186	1 242	1 178	1 160	1 14	1 112	1 150	1 188
200			200	1 268	1 158	1 186	1 248	1 170	1 160	1 14	1 124	1 150	1 208
204	309	349	200	1 282	1 150	1 202	1 220	1 170	1 160	1 18	1 124	1 154	1 208
204			206	1 268	1 158	1 184	1 250	1 170	1 184	1 22	1 112	1 158	1 212
206	1	2	218	0 276	0 156	0 186	0 252	0 186	0 174	0 18	0 124	1 150	0 212
206			200	0 268	0 148	0 184	0 248	0 170	0 160	0 14	0 124	1 146	0 188
207	277	278	200	1 268	1 154	1 194	1 220	1 170	1 178	1 18	1 128	1 146	1 192
207			204	1 268	1 158	1 184	1 230	1 178	1 176	1 22	1 112	1 154	1 216
209	0	0	200	1 268	0 154	1 186	0 242	1 170	1 172	1 10	1 126	0 146	1 188
209			200	1 256	0 150	1 184	0 254	1 186	1 172	1 16	1 124	0 158	1 188
213	0	0	216	0 272	1 150	1 186	0 250	1 170	1 160	1 10	1 124	1 150	1 212
213			200	0 282	1 150	1 184	0 238	1 170	1 180	1 14	1 124	1 150	1 196
214	460	459	202	1 268	1 158	1 200	1 220	1 178	1 176	1 10	1 126	1 150	1 188
214			216	1 276	1 154	1 186	1 242	1 170	1 176	1 18	1 124	1 150	1 188
215	1	270	218	1 276	1 160	0 186	1 242	1 178	1 174	1 14	1 124	1 154	0 192
215			200	1 268	1 154	0 186	1 230	1 170	1 160	1 14	1 124	1 150	0 188
216	0	259	204	1 278	0 156	1 186	0 230	1 178	1 170	1 16	1 130	1 154	0 216
216			200	1 268	0 162	1 184	0 252	1 170	1 160	1 14	1 128	1 150	0 192
218	273	272	200	1 268	1 162	1 186	1 220	1 186	1 172	0 10	1 112	1 150	1 212
218			200	1 268	1 158	1 186 184	1 246	1 170	1 174	0 20	1 124	1 158	1 188
219	0	0	202	1 268	1 154	1 186 186	1 230	1 178	1 172	1 10	1 124	1 154	1 188
219			200	1 256	1 168	1 184	1 250	1 170	1 174	1 16	1 126	1 146	1 188
220	267	2	216	1 268	1 152	1 186	1 230	1 178	1 176	1 10	1 126	1 154	1 208
220			200	0 268	1 154 148	1 186	1 232	1 178	1 172	1 10	1 126	1 142	1 212
221	0	0	202	1 268	1 160 154	1 184	0 250	0 178	0 174	0 18	0 124	1 154	1 216
221			202	1 268	1 154	1 186	0 250	1 170	1 172	1 10	1 126	1 158	1 188
223	0	0	202	1 280	0 148	0 184	0 256	0 186	0 174	0 18	1 124	1 158	0 212
223			202	0 268	0 154	0 186	0 252	0 178	0 172	0 18	0 124	1 146	0 208
225	264	2	200	1 268	1 164	1 186	1 230	1 178	0 172	0 26	0 124	1 158	1 216
225			200	1 268	1 158	1 186	1 246	1 170	0 168	0 10	0 124	1 158	1 188
225			200	1 268	1 158	1 186	0	0 178	0	0 172	10 0 124	1 150	0 188
226	1	2	202	1 268	1 154	0 186	0 230	0 178	0 172	0 172	16 0 124	0 142	0 188
226			202	1 256	0 148	0 184	0 254	0 170	0	0 174	1 128	1 150	1 192
228	1	260	200	1 268	1 150	1 202	1 220	1 170	1 174	0 18	1 128	1 150	1 192
228			200	1 268	1 158	1 186	1 242	1 178	1 172	0 18	1 124	1 158	1 208

Affected haplotypes

18p	PAN	MAN	ca212	1140	59	ca231	ta201	at201	PDca225	w3442	ca213	ga201	ga203	
229	257	2	200	1 268	0 154	1 186	1 244	1 170	1 174	1 10	1 126	1 150	1 192	1
229			216	1 256	0 158	1 186	1 244	1 186	1 174	1 24	1 124	1 146	1 216	1
230	0		202	1 268	1 160	1 186	1 230	1 170	1 172	0 18	1 122	1 150	1 208	1
230		0	202	1 268	1 158	1 186	1 248	1 170	1 160	0 12	1 124	1 150	1 216	1
231	299	298	216	1 268	1 158	1 186	1 220	1 170	1 172	1 20	1 124	1 150	1 204	1
231			218	1 268	1 158	1 186	1 244	1 170	1 174	1 22	1 126	1 150	1 204	1
232	1	310	206	1 268	1 150	1 186	1 222	1 170	1 172	1 20	1 124	1 154	0 188	1
232			200	1 268	1 158	1 186	1 230	1 170	1 178	1 10	1 126	1 150	0 188	1
234	1	261	200	1 268	1 148	1 184	1 252	1 170	1 174	1 10	1 126	1 162	1 208	1
234			200	1 268	1 158	1 186	1 262	1 170	1 174	1 24	1 126	1 150	1 192	1
235	0	0	200	1 276	0 150	1 186	0 248	1 170	1 172	1 10	1 112	1 154	0 192	1
235			202	1 268	0 156	1 184	0 214	1 170	1 174	1 22	1 124	1 150	0 192	1
237	0		200	1 268	1 158	1 186	1 214	1 178	1 172	1 16	1 126	0 150	1 208	1
237		0	200	1 268	1 154	1 186	1 230	1 186	1 172	1 16	1 124	0 154	1 208	1
238	456	457	202	1 268	1 154	1 186	1 230	1 178	1 172	1 10	1 128	1 150	1 208	1
238			200	1 268	1 158	1 186	1 230	1 170	1 178	1 14	1 112	1 150	1 188	1
239	312	2	218	1 268	1 160	1 186	0 248	1 170	1 172	1 16	1 124	1 154	0 208	0
239			200	1 268	1 158	1 184	0 242	1 178	1 172	1 18	1 124	0 154	0 188	0
240	1	2	200	1 268	1 158	0 186	1 242	0 178	1 172	1 18	1 128	0 146	0 188	0
240			200	1 268	1 148	0 186	1 230	0 178	1 172	1 18	1 124	0 150	0 188	0
241	1	342	216	1 268	1 158	184	0 246	1 170	1 172	1 20	1 126	0 142	1 188	1
241			200	1 268	1 158	186	0 250	1 170	1 172	1 10	1 124	0 150	1 192	1
242	0	0	216	1 268	1 156	0 186	244	244	1 170	1 174	0 126	0 126	1 150	1 188
242			200	1 268	1 154	0 186	1 230	1 170	0 172	0 10	1 124	1 150	1	188
243	347	274	200	1 268	1 154	1 186	1 230	1 178	0 160	0 38	1 124	1 150	1	208
243			218	1 268	1 150	1 186	1 252	1 170	0 160	0 38	1 126	1 146	1 216	1
245	0	0	200	1 268	1 154	1 186	1 232	1 178	0 172	1 10	1 124	1 150	1 192	1
245			202	1 268	1 150	1 186	1 242	1 170	0 172	1 16	1 126	1 150	1 188	1
246	1	262	204	1 268	1 158	1 186	1 246	1 178	0 172	1 16	1 122	1 150	1 216	1
246			202	0 268	1 154	1 186	1 242	1 170	0 172	1 22	1 124	1 158	1 188	1
247	303	302	202	0 268	1 154	1 186	1 230	1 178	1 174	1 10	1 124	1 150	1 216	1
247			200	1 268	1 154	1 186	1 242	1 170	1 176	1 10	1 126	1 150	1 216	1
248	334	333	200	1 268	1 154	1 184	1 232	1 170	1 160	1 20	1 112	1 150	1	0
248			202	1 268	1 154	1 186	1 244	1 170	1 174	1 16	1 112	1 146	1	0

Affected haplotypes

18p	PAN	MAN	ca212	1140	59	ca231	ta201	at201	PDca225	w3442	ca213	ga201	ga203	
249	1	2	200	0 268	0 154	0 186	1 230	0 194	0 172	0 10	0 124	1 150	1 188	1
249			216	0 256	0 148	0 186	1 246	0 178	0 174	0 16	0 124	1 150	1 188	1
251	301	300	216	1 272	1 150	1 184	1 250	1 170	1 160	1 10	1 124	1 150	1 212	1
251			216	1 268	1 158	1 186	1 244	1 186	1 174	1 20	1 124 124	1 150	1 188	1
252	1	285	200	0 268	1 154	1 186	1 230	1 178	1 172	1 10	1 126	0 150	1 188	1
252			204	0 268	0 158	1 186	1 246	1 170	1 160	1 18	1	0 150	1 216	1
253	1	258	216	0 268	1 160	1 186	1 228	1 170	1 160	1 16	1 124	1 150	1 188	1
253			200	0 268	1 154	1 186	1 230	1 178	1 160	1 16	1 126	1 150	1 216	1
254	467	2	202	1 268	1 160	1 186	1 230	1 170	0 172	1 18	1 122	1 150	1 208	0
254			200	1 268	1 154	1 186	1 230	1 178	0 172	1 10	1 124	1 142	1 188	0
265	1	266	216	1 272	1 150	1 184	1 250	1 170	1 160	1 10	1 126	0 150	1 212	1
265			202	1 268	1 154	1 186	1 230	1 178	1 172	1 10	1 124	0 150	1 188	1
311	1	485	216	1 268	1 154	1 186	1 244	1 170	1 160	1 10	1 126	1 150	1 188	1
311			200	1 268	1 162	1 186	1 242	1 170	1 174	1 10	1 124	1 158	1 208	1
314	348	313	200	1 268	1 148	1 184	1 248	1 186	1 168	1 18	0 124	1 150	1 208	1
314			216	1 268	1 162	1 184	1 250	1 170	1 172	1 10	0 126	1 150	1 188	1
316	1	317	214	1 268	1 154	1 186	1 230	1 178	1 172	1 10	1 124	1 150	1 208	0
316			200	1 268	1 154	1 186	1 242	1 170	1 172	1 10	1 126	1 150	1 188	0
319	318	2	202	0 272	0 158	0 184	0 244	0 178	0 184	0 10	1 126	0 150	1 188	1
319			200	0 256	0 154	0 186	0 244	0 170	0 174	0 10	1 112	0 150	1 188	1
321	1	320	202	0 268	0 158	0	0	0 178	1 178	0 18	1 128	0	0	0
321			200	0 268	0 154	0	0	0 170	1 172	0 10	1 124	0	0	0
324	0	0	202	1 268	1 158	1 186	1 232	1 178	1 172	0 24	1 112	1 150	1 212	1
324			216	1 268	1 150	1 196	1 220	1 170	1 160	0 18	1 128	1 154	1 208	1
326	325	336	206	1 280	1 152	1 198	1 232	1 170	1 172	1 16	1 124	1 150	1 188	1
326			202	1 268	1 154	1 186	1 232	1 178	1 160	1 16	1 132	1 150	1 192	1
329	1	330	200	1 268	1 154	0 186	1 248	1 170	1 160	1 14	1 128	1 150	1 188	1
329			206	1 268	1 148	0 186	1 234	1 170	1 172	1 22	1 124	1 150	1 208	1
211	1	2	200	0 268	0 154	0 186	0 230	0 178	1 172	1 10	0 126	0 150	0 188	1
211			204	0 268	0 148	0 198	0 252	0 178	1 172	1 18	0 112	0 154	0 188	1
353	1	352	218	1 280	1 148	1 186	1 246	1 170	1 160	1 18	1 132	1 154	1 192	1
353			200	1 268	1 148	1 186	1 246	1 170	1 172	1 18	1 112	1 146	1 192	1
356	362	2	216	1 268	1 154	1 186	0 248	1 178	0 172	1 10	0 124	1 150	1 208	1
356			204	1 268	1 164	1 190	0 232	1 170	0 172	1 18	0 126	1 150	1 216	1

18p	PAN	MAN	ca212	1140	59	ca231	ta201	at201	PDca225	w3442	ca213	ga201	ga203	18p
357	1	358	0 202	0 268	0 154	0 186	1 232	1 178	1 160	1 10	1 128	0 150	1 196	1
357			0 214	0 278	0 158	0 186	1 248	1 178	1 184	1 10	1 124	0 150	1 208	1
359	378	365	1 202	1 268	1 154	1 186	1 230	1 178	1 172	1 10	1 126	1 154	1 188	1
359			1 202	1 272	1 158	1 184	1 244	1 178	1 184	1 10	1 112	1 150	1 188	1
367	1	366	1 202	1 268	1 154	1 186	1 232	1 178	1 172	1 10	1 126	1 158	0 208	1
367			1 202	1 268	1 154	1 186	1 242	1 178	1 172	1 10	1 112	1 142	0 208	1
372	1	370	1 200	1 268	1 154	1 186	0	0	0 172	1 10	1 124	0 150	1	0
372			1 216	1 268	1 148	1 184	0	0	0 174	1 10	1 126	0 150	1	0
384	389	2	1 202	1 268	1 156	1 186	1 246	1 170	1 174	1 10	1 126	1 150	1 188	1
384			1 202	1 268	1 154	1 186	1 250	1 170	1 174	1 10	1 126	1 158	1 188	1
409	408	410	1 216	1 268	1 148	1 200	1 220	1 170	1 184	1 24	1 132	1 154	1 208	1
409			1 202	1 268	1 154	1 186	1 230	1 178	1 172	1 10	1 124	1 150	1 216	1
435	1	433	1 200	1 280	1 148	1 184	1 252	1 178	1 178	0 22	1 126	1 150	1 204	1
435			1 202	1 268	1 156	1 194	1 220	1 170	1 172	0 22	1 126	1 150	1 204	1
443	1	444	1 206	1 280	1 148	1 186	1 246	1 178	1 176	0 14	1 128	1 154	0 192	1
443			1 202	1 256	1 154	1 186	1 230	1 178	1 172	0 10	1 124	1 150	0 188	1
458	1	551	1 200	1 268	1 162	1 186	1 230	1 178	1 172	1 22	1 126	1 150	1 208	0
458			1 200	1 268	1 154	1 186	1 234	1 178	1 172	1 12	1 128	1 154	1 188	0
488	1	508	1 216	1 268	1 160	1 186	1 232	1 170	1 172	1 18	1 122	1 150	1 208	1
488			1 216	1 268	1 160	1 184	1 232	1 170	1 172	1 18	1 122	1 150	1 208	1
501	528	527	1 200	1 268	1 154	1 186	1 230	1 178	1 176	1 10	1 126	1 150	1 216	1
501			1 206	1 268	1 154	1 186	1 244	1 170	1 172	1 16	1 126	1 154	1 208	1
505	1	502	1 202	1 268	1 158	1 186	1 244	1 170	1 172	1 22	1 126	1 150	1 188	1
505			1 200	1 268	1 158	1 186	1 244	1 170	1 172	1 16	1 126	1 150	1 188	1
516	1	517	1 202	0 268	1 158	0	0	0	0	0 10	1 128	0	0 208	0
516			1 200	0 268	1 148	0	0	0	0	0 10	1 124	0	0 200	0
537	532	534	1 202	1 256	1 154	1 186	1 230	1 178	0 172	1 10	1 124	1 150	1 188	1
537			1 216	1 268	1 154	1 184	1 230	1 170	0 172	1 10	1 126	1 146	1 216	1
531	1	529	1 202	1 268	1 150	1 184	1 254	1 170	1 160	1 18	0 124	1 158	1 188	1
531			1 200	0 268	1 154	1 186	1 244	1 170	1 174	1 10	0 124	1 150	1 192	1
574	0	0	1 206	1 274	0 152	1 194	1 236	1 170	1 174	0 18	1 124	1 150	0 192	0
574			1 200	0 268	0 148	1 184	1 252	1 186	1 172	0 18	1 124	1 146	0 188	0
578	576	579	1 202	1 280	1 154	1 186	1 214	1 170	1 174	1 18	1 124	1 150	1 192	1
578			1 202	1 268	1 154	1 186	1 230	1 178	1 172	1 10	1 124	1 162	1 188	1

18p	PAN	MAN	ca212	1140	59	ca231	ta201	at201	PDca225	w3442	ca213	ga201	ga203	
587	580	582	1 202	1 256	1 158	1 186	1 248	1 170	1 174	1 16	1 124	1 150	1 208	1
587			202	1 268	1 154	1 186	1 244	1 170	1 172	1 10	1 132	1 150	1 208	1
361		360	204	0 270	1 158	1 186	1 244	1 170	1 172	1 10	1 126	1 150	1 208	1
361	1		202	0 276	1 148	1 186	1 236	1 170	1 172	1 20	1 128	1 150	1 212	1
368	0	0	204	1 268	1 164	1 186	1 242	0 178	0 172	1 10	1 124	0 150	1 192	0
368			202	1 256	1 154	1 186	1 230	0 170	0 160	1 10	1 126	0 154	1 212	0
374	1	2	1 200	1 268	1 154	1 186	1 230	1 178	1 174	0 10	1 126	0 150	0 188	0
374			1 200	1 268	1 154	1 186	1 230	1 178	1 160	0 10	1 124	0 142	0 212	0
399	0	0	1 202	1 268	1 148	1 184	1	0 170	1 174	0 16	1 124	1 142	1 188	1
399			1 204	1 272	1 158	1 186	1	0 178	1 172	0 18	1 126	1 150	1 200	1
411	1	2	1 216	0 270	0 164 184	0 184	0 252	0 170	0 174	0 18	0 124	1 150	0 188	0
411			202	0 268	0 154 186	0 186	0 230	0 178	0 160	0 10	0 124	1 142	0 188	0
413	414	412	1 200	1 268	1 158	1 186	1 230	1 178	1 178	1 18	1 112	1 150	1 188	1
413			202	1 280	1 148	1 186	1 244	1 170	1 176	1 24	1 126	1 154	1 188	1
236	697	698	1 216	1 268	1 158	1 186	1 220	1 170	1 172	1 20	1 124	1 150	1 204	0
236			1 216	1 268	1 158	1 186	1 220	1 170	1 172	1 20	1 124	1 150	1 204	1
421	0	0	1 200	1 268	1 148	1 184	0 252	1 170	1 174	1 10	1 126	1 150	1 188	1
421			202	1 268	1 152	1 186	0 242	1 190	1 172	1 10	1 126	1 150	1 188	1
424	1	2	1 200	1 268	1 158	0 194	0 220	0 170	0 178	0 24	0 128	0 150	0 208	0
424			200	1 268	1 154	0 186	0 232	0 178	0 160	0 18	0 112	0 146	0 192	0
452	1	2	1 202	0 256	0 148	0 184 182	1 252	0 170	1 174	0 16	1 124	1 158	1 188	1
452			200	0 268	0 154	1 186 186	1 250	0 170	1 160	0 10	0 124	1 150	1 188	1
473		472	1 202	1 268	1 162	1 186	1 246	1 170	1 180	1 22	1 126	1 146	1 212	1
473	1		218	1 268	1 148	1 186	1 244	1 170	1 160	1 10	1 124	1 150	1 188	1
484	482		1 200	1 276	1 148	1 182	0 246	1 170	1 174	1 14	1 124	1 150	1 188	1
484		2	206	1 256	1 154	1 186	0 244	1 170	1 174	1 10	1 126	1 150	1 212	1
487	1	486	1 200	1 268	1 158	1 190	0 248	1 170	1 174	1 12	0 126	1 158	1 192	1
487			202	1 278	1 148	1 186	0 246	1 182	1 180	1 10	0 112	1 150	1 208	1
331	1	476	1 202	0 268	1 154	1 186	1 234	1 178	0 172	1 24	1 126	0 158	0 212	0
331			200	0 268	1 154	1 186	1 230	1 170	0 172	1 10	1 112	0 150	0 188	0
489	0	0	1 202	1 268	1 158	1 186	1 244	1 170	1 172	1 10	1 124	1 150	1 204	1
489			200	1 268	1 148	1 202	1 220	1 178	1 172	1 10	1 132	1 162	1 208	1
498	1	635	1 200	1 268	1 160	1 186	1 246	1 170	1 172	1 14	1 122	1 150	1 208	1
498			200	1 268	1 164	1 186	1 246	1 170	1 172	1 18	1 112	1 150	1 188	1

Affected haplotypes

18p	PAN	MAN	ca212	1140	59	ca231	ta201	at201	PDca225	w3442	ca213	ga201	ga203	
566	0	0	216	1 268	1 148	1 202	1 220	0 178	1 174	1 10	1 124	1 150	1 212	1
566			202	1 268	1 154	186	1 230	0 178	1 172	1 10	128	1 150	1 208	1
514	1	2	202	0 268	1 154	1 186	0 230	1 178	1 172	0 10	1 124	1 154	0 192	1
514	1		200	0 268	1 154	184	0 230	178	1 168	0 10	124	1 146	0 192	1
536	1	633	202	1 270	0 148	1 184	1 254	0 170	1 168	0 16	0 132	0 162	1 212	0
536			200	0 268	0 154	1 186	1 252	1 178	1 172	0 24	124	0 154	1 188	0
605	1	2	216	0 268	1 158	0 198	0 244	170	0 172	1 16	0 124	158	200	0
605			200	1 268	1 150	0 186	0 220	178	0 172	1 10	124	150	188	0
540	539	562	200	1 268	1 154	1 186	1 230	1 178	1 172	1 10	1 124	1 150	1 216	1
540			216	1 268	1 148	1 186	1 230	1 194	1 172	1 22	1 112	1 154	1 212	1
684	1	730	202	1 268	1 158	1 186	1 232	1 178	1 160	1 24	1 112	1 150	1 212	1
684			200	0 268	1 154	1 186	1 244	1 170	1 160	1 10	1 126	1 150	1 188	1
608	1	2	206	0 268	1 156	0 192	0 244	0 170	0 178	0 22	0 126	1 150	1 204	0
608			202	0 268	1 154	0 186	0 220	0 170	0 174	0 10	0 126	1 150	1 188	0
637	1	638	216	1 268	1 162	0 186	1 250	1 182	1 172	1 10	1 124	1 142	0 208	1
637			200	1 268	1 154	0 186	1 230	178	1 172	1 10	124	1 150	0 212	1
649	647	646	200	1 268	1 154	1 186	1 230	1 178	1 172	1 10	1 124	1 150	1 188	1
649			200	1 268	1 154	1 186	1 230	1 178	1 172	1 10	1 124	1 150	1 188	1
653	1	652	200	1 270	1 162	1 184	1 250	1 170	1 180	1 10	1 112	1 154	1 188	1
653			200	1 280	0 160	0 184	1 230	1 178	1 184	1 20	1 128	1 154		0
491	1	2	204	1 268	1 158	0 186	1 230	1 178	1 168	1 22	1 112	1 150		0
491			202	0 268	1 148	0 194	0 256	0 178	0 180	0 22	0 124	1 158	0 204	0
493	1	2	202	0 282	0 158	0 184	0 230	0 170	0 174	0 10	0 124	1 154	0 188	0
493			200	0 268	0 156	0 186	0 242	1 170	1 174	0 16	0 124	1 158	0 212	0
506	1	2		0	0	0	1 242	1 170	1 172	0 14	0 124	1 150	0 204	0
506				0	0	0	0	0	0	0	0	0 150	1	0
661	660	662	200	1 278	1 156	1 198	1 220	1 170	1 174	1 20	1 126	1 154	1 204	1
661			200	1 268	1 148	1 184	1 250	1 186	1 174	1 18	1 120	1 150	1 188	1
667	666	668	202	1 268	1 154	1 186	1 214	1 170	1 160	1 22	1 124	1 146	1 212	1
667			202	1 268	1 162	1 186	1 246	1 178	1 172	1 18	1 112	1 158	1 188	1
669	670	671	202	1 268	1 162	1 186	1 258	1 186	1 174	1 18	1 126	1 150	1 188	1
669			200	1 268	1 154	1 186	1 244	1 170	1 160	1 10	1 126	1 150	1 188	1
676	1	678	202	0 268	1 158	1 190	1 244	1 178	1 172	1 16	1 126	1 158	1 188	1
676			200	0 280	1 148	1 184	1 252	1 178	1 172	1 22	1 126	1 150	1 216	1

Affected haplotypes

18p	PAN	MAN	ca212		1140	59	ca231	ta201	at201	PDca225	w3442	ca213	ga201	ga203	
681	1	2	202	0	256	0 162	0 186	1 260	0 186	0 174	0 18	0 126	0 150	1 192	0
681			200	0	268	0 154	0 186	1 230	0 178	0 172	0 10	0 124	0 150	1 188	0
351	354	2	202	1 268		1 154	1 186	1 230	1 178	1 172	16	1 126	1 150	1 188	1
351			216	1 268		1 156	1 186	1 244	1 186	1 174	1 24	1 124	1 150	1 208	1
355	1	2	216	0 272		0 158	0 190	0 248	170 0	0 172	1 18	0 126	0 158	0 188	1
355			204	0 268		0 152	0 186	0 244	178 0	0 172	1 10	0 124	0 150	0 188	1

60015v1/PA

ca219	1105	ca209	ca202	1146	166d05	476	
241	1 85	1 173	1 192	1 272	1 312	1 271	1
233	1 99	1 181	1 196	1 270	1 304	1 271	1
241	1 85	1 173	1 182	1 274	0 312	1 273	1
245	1 103	1 177	1 194	1 270	0 308	1 267	1
241	1 85	1 173	1 198	0 274	1 308	0 275	0
241	1 85	1 173	1 194	0 274	1 304	0 271	0
241	1 87	1 173	1 182	1 272	1 300	1 271	1
235	1 101	1 181	1 196	1 274	1 312	1 271	1
235	1 85	1 173	1 182	1 274	1 312	1 271	1
243	1 85	1 173	1 192	1 274	1 316	1 267	1
245	1 103	1 177	1 194	0 274	0 312	1 271	1
235	1 91	1 181	1 182	0 270	0 316	1 271	1
241	1 85	1 173	1 182	1 274	1 312	1 271	1
241	1 103	1 177	1 196	1 274	1 312	1 271	1
241	1 85	1 173	1 196	0 270	1 300	1 271	0
235	1 85	1 181	1 190	0 274	1 312	1 267	0
235	1 81	1 173	1 182	1 274	1 324	1 271	0
223	1 83	1 173	1 192	1 274	1 300	1 267	0
245	1 103	1 177	1 196	1 274	1 312	1 271	1
241	1 85	1 173	1 182	1 270	1 312	1 265	1
241	1 105	0 173	1 196	1 270	1 304	1 267	1
241	1 101	0 173	1 196	1 270	1 308	1 271	1
241	1 87	0 173	1 192	1 274	1 312	1 271	1
241	1 85	1 177	1 196	1 274	1 304	1 267	1
245	1 97	1 177	1 194	1 274	1 312	0 271	1
235	1 99	1 181	1 198	1 274	1 300	0 271	1
241	0 95	0 181	0 198	0 274	1 320	0 273	0
235	0 85	0 173	0 196	0 274	1 308	0 271	0
235	1 101	0 181	0 196	1 272	1 312	1 271	1
235	1 85	0 173	0 200	1 274	1 308	1 271	1
241	0 85	0 173	1 200	0 274	0 312	0 271	0
243	0 101	0 173	1 196	1 270	0 304	0 267	0
241	1 85	1 173	1 182	1 274	1 316	1 271	0
241	1 99	1 173	1 200	1 274	1 300	1 269	0

60015v1/PA

ca219	1105	ca209	ca202	1146	166d05	476	
241	1 85	1 177	1 196	1 270	1 304	1 271	1
245	1 99	1 177	1 192	1 274	1 308	1 265	1
245	1 97	1 177	1 196	0 274	1 304	1 275	0
245	1 99	1 177	1 192	0 270	1 308	1 267	0
243	1 103	1 175	1 198	1 274	1 300	1 271	1
245	1 85	1 173	1 194	1 274	1 312	1 271	1
235	1 101	0 181	0 196	1 270	1 316	1 267	1
235	1 85	0 173	0 196	1 274	1 300	1 271	1
241	1 85	1 173	1 200	0 270	1 304	1 273	1
241	1 85	1 177	1 198	0 274	1 308	1 271	1
241	0 101	0 177	1 182	1 274	1 312	1 273	1
235	0 85	0 177	1 190	1 274	1 300	1 275	1
241	1 85	1 173	1 194	1 274	1 308	1 271	1
239	1 85	1 173	1 196	1 270	1 308	1 271	1
245	0 85	1 177	1 198	1 274	1 320	1 271	1
241	0 85	1 173	1 196	1 274	1 308	1 265	1
241	1 99	0 177	1 198	1 270	1 312	1 271	0
241	1 85	0 173	1 182	1 270	1 312	1 263	0
241	0 101	0 187	0 200	0 270	1 312	0 271	1
235	0 85	0 173	0 182	0 270	1 300	0 271	1
241	0 101	1 181	0 196	1 274	1 308	0 275	0
235	0 83	1 173	0 196	1 270	1 304	0 267	0
241	1 85	1 173	1 196	1 270	1 300	1 275	1
235	1 101	1 181	1 196	1 272	1 300	1 271	1
241	1 85	1 173	1 182	1 270	1 300	1 271	1
239	1 103	1 173	1 194	1 274	1 312	1 271	1
241	1 85	1 173	1 194	1 274	1 316	1 271	1
241	1 85	1 173	1 196	1 270	1 308	1 271	1
241	1 105	1 173	1 196	1 274	1 312	0 271	0
235	1 101	1 181	1 182	1 270	1 300	0 267	0
243	1 85	1 173	1 196	1 274	1 300	1 271	1
241	1 85	1 173	1 190	1 270	1 316	1 271	1
241	1 85	1 177	1 196	1 274	1 304	1 271	0
241	1 85	1 173	1 196	1 270	1 312	1 267	0

Affected haplotypes

ca219	1105	ca209	ca202	1146	166d05	476
1 241	85	0 173	1 192	0 272	0 312	0 273
1 241	103	0 173	1 182	0 270	0 304	0 267
1 245	1 103	1 181	0 194	1 270	1 312	1 271
1 235	1 101	1 177	0 202	1 274	1 312	1 271
0 241	0 103	1 181	0 196	1 276	0 304	1 271
0 235	0 101	1 173	0 208	1 274	0 300	1 267
1 241	1 85	1 173	1 198	1 274	1 304	1 271
1 241	1 85	1 173	1 190	1 274	1 312	1 271
1 245	1 97	1 177	1 196	1 274	1 304	0 275
1 235	1 99	1 181	1 196	1 270	1 312	0 271
1 245	1 103	1 177	1 194	1 274	1 308	1 271
1 245	1 85	1 173	1 192	1 274	1 300	1 267
1 235	1 101	1 181	1 196	0 272	1 320	0 271
1 241	1 85	1 173	1 184	0 274	1 312	0 269
1 245	1 85	1 177	1 196	1 274	1 312	1 271
1 235	1 101	1 181	1 182	1 270	1 312	1 269
0 241	0 103	0 181	0 190	1 274	0 304	0 271
0 235	0 101	0 173	0 190	1 274	0 312	0 267
1 241	1 101	1 181	0 196	0 274	1 312	1 271
1 235	1 103	1 173	0 192	0 274	1 300	1 271
0	0 101	1	0	0 270	1 304	1
0	0 85	1	0	0 272	1 300	1
1 241	1 85	1 173	1 194	0 274	0 312	1 269
1 241	1 101	1 177	1 182	0 270	0 312	1 267
1 241	1 85	1 173	1 182	1 276	1 320	1 269
1 241	1 85	1 173	1 194	1 270	1 300	1 271
1 241	1 85	1 173	1 200	1 272	0 304	1 271
1 241	1 85	1 181	1 190	1 274	1 316	1 271
1 241	1 85	1 181	1 182	0 274	1 312	0 267
0 235	0 85	1 181	1 196	0 274	0 316	0 263
1 235	1 81	1 179	1 196	0 274	1 312	1 269
1 235	1 85	1 179	1 182	0 274	1 312	1 271
0 235	85	0 181	1 194	1 274	1 300	0 275
1 241	101	0 173	1 196	1 270	1 300	0 271

ca219	1105		ca209		ca202		1146		166d05		476	
243	0	103	0	177	0	196	0	274	0	308	1	271
241	0	85	0	173	0	190	0	270	0	312	1	265
235	1	99	1	181	1	196	1	274	1	308	0	271
235	1	101	1	181	1	196	1	272	1	308	0	267
241	1	85	1	177	0	192	1	270	1	316	0	269
245	1	85	1	173	0	184	1	274	1	308	0	265
241	1	99	1	177	1		0	274	0	308	1	267
241	1	105	1	173	1		0	270	0	300	1	271
241	0	103	1	181	0	190	1	274	1	312	1	271
235	0	97	1	173	0	198	1	270	1	300	1	267
241	1	99	1	177	0	182	1	274	1	308	1	271
241	1	85	1	173	0	196	1	274	1	300	1	271
245	1	85	1	177	1	182	1	274	1	312	1	273
245	1	85	1	177	1	182	1	274	1	312	1	267
241	1	85	1	175	1	196	1	274	1	320	1	261
241	1	101	1	173	1	196	1	270	1	304	1	267
241	0	85	1	173	1	186	1	270	1	316	1	269
239	0	85	1	173	1	182	1	270	1	312	1	273
235	1	101	1	181	1	184	1	274	1	324	1	269
235	1	101	1	181	1	184	1	274	1	324	1	269
241	1	85	1	173	1	190	1	274	0	316	1	271
245	1	101	1	175	1	196	1	270	0	308	1	271
241	1	85	1	173	1	196	0	270	1	316	1	267
243	1	85	1	173	1	192	0	274	1	308	1	267
241	0	99	0	181	0	196	0	274	1	312	0	271
235	0	85	0	173	0	192	1	274	1	312	0	267
241	1	101	1	173	1	196	1	270	1	304	1	267
241	1	85	1	173	1	194	1	270	1	312	1	267
241	1	99	1	173	1	192	1	274	1	312	1	271
225	1	83	1	173	1	192	1	270	1	308	1	269
241	1	85	0	173	1	182	1	274	0	312	0	271
241	1	85	1	181	1	182	1	270	0	308	0	269
245	1	103	1	177	1	196	0	270	1	304	1	267
241	1	105	1	173	1	192	0	274	1	316	1	271

Affected haplotypes

ca219	1105		ca209		ca202		1146		166d05		476	
	0	85	1	173	1	190	1	274	1	312	1	271
	0	101	1	181	1	198	1	272	1	312	1	263
241	1	99	1	177	0	198	1	274	1	312	1	271
241	1	101	1	173	0	196	1	276	1	304	1	265
241	1	85	1	173	1	196	1	270	1	304	1	271
241	1	85	1	173	1	190	1	270	1	312	1	271
241	1	85	0	173	1	200	0	270	1	312	0	271
241	1	101	0	173	1	186	0	270	1	304	0	271
243	1	85	1	173	1	200	1	274	1	312	1	271
235	1	95	1	181	1	196	1	274	1	312	1	271
241	0	85	1	173	1	200	1	274	1	312	0	271
243	0	85	1	173	1	200	1	274	1	308	0	271
241	1	85	1	173	1	194	1	274	1	300	1	275
241	1	85	1	181	1	196	1	274	1	300	1	271
243	1	103	1	175	1	198	1	274	1	300	1	271
243	1	103	1	175	1	196	1	274	1	308	1	271
235	1	97	1	181	1	196	1	274	1	300	1	271
235	1	99	1	181	1	192	1	270	1	312	1	267
241	0	101	0	181	0	194	0	274	0	308	0	271
235	0	85	0	173	0	182	0	272	0	300	0	267
243	0	103	0	173	1	196	1	274	1	308	0	269
241	0	85	0	173	1	196	1	274	1	304	0	267
241	1	87	1	177	0	196	1	274	0	312	1	271
241	1	85	1	173	0	194	1	270	0	300	1	275
241	1	105	1	173	1	196	1	274	0	312	1	271
241	1	85	1	173	1	192	1	270	0	312	1	267
243	0	85	1	173	1	198	1	270	1	304	1	271
241	0	85	1	173	1	196	1	274	1	312	1	271
241	1	85	1	173	1	196	1	274	1	308	0	271
241	1	85	1	173	1	182	1	274	1	320	0	265
245	0	85	1	177	0	198	1	270	1	304	0	271
241	0	85	1	173	1	194	1	274	1	312	0	267
241	1	103	1	177	1	196	1	270	1	316	1	267
235	1	99	1	181	1	192	1	270	1	312	1	271

ca219		1105		ca209		ca202		1146		166d05		476	
1	245	1	105	0	177	1	196	1	274	1	300	1	267
1	245	1	85	0	177	1	198	1	274	1	320	1	271
1	241	1	97	0	177	0	196	1	274	0	304	0	271
1	241	1	85	0	173	0	196	1	272	0	300	0	271
1	241	1	99	0	177	0	196	1	274	1	312	1	271
1	241	1	85	0	173	0	182	1	274	1	312	1	271
0	243	0	85	1	173	1	200	0	274	1	308	1	271
0	235	0	85	1	173	1	194	0	274	1	308	1	271
1	241	1	85	1	173	1	190	1	274	0	312	1	267
1	235	1	85	1	173	1	196	1	272	0	316	1	267
1	241	0	85	1	181	0	196	1	274	0	312	1	269
0	235	0	101	1	173	0	196	1	272	0	300	1	271
0	245	0	101	0	173	0	182	0	274	1	312	1	273
0	241	0	85	0	177	0	190	0	274	1	312	1	267
1	239	1	85	1	173	1	190	1	270	1	300	1	271
1	241	1	85	1	173	1	198	1	274	1	304	1	271
0	241	0	85	1	173	1	198	1	270	1	304	1	271
0	243	0	85	1	173	1	182	1	274	1	312	1	269
1	245	1	85	1	179	1	196	1	270	1	308	1	271
1	241	1	85	1	173	1	196	1	270	1	304	1	265
1	241	0	103	0	173	1	198	0	274	1	308	0	269
1	235	0	81	0	173	1	196	0	274	1	308	0	265
1	241	1	103	0	177	0	196	0	270	0	308	0	271
1	241	1	85	0	173	0	190	0	270	0	300	0	269
0	245	0		0		0		0		0		0	
0	241	0		0		0		0		0		0	
1	235	1	81	1	173	1	196	1	276	1	300	1	271
1	241	1	85	1	173	1	196	1	274	1	308	1	265
1	245	1	103	1	177	1	196	1	270	1	308	1	271
1	235	1	97	1	181	1	192	1	274	1	312	1	271
1	241	1	101	0	173	1	192	1	274	1	316	1	271
1	235	1	85	0	181	1	190	1	270	1	312	1	271
1	235	1	97	1	181	1	198	1	274	1	312	0	271
1	243	1	103	1	173	1	182	1	274	1	308	0	273

ca219	1105	ca209	ca202	1146	166d05	476	
241	1 103	0 177	0 190	0 270	0 304	0 271	1
241	1 85	0 173	0 196	0 274	0 312	0 271	1
241	1 101	1 173	1 192	1 274	1 312	1 271	0
245	1 105	1 177	1 194	1 274	1 320	1 267	0
241	1 103	0 177	0 196	1 274	0 316	0 267	1
241	1 85	0 173	0 196	1 270	0 304	0 267	1

Figure 9

nontransmitted chromosomes

ERSN	KID	sava5	ca211	ca212	1140	59	ca231	ta201	at201	ca225	w3442	ca213	ga201	ga203
279	200	235	1 193	1 216	1 268	1 148	1 186	1 246	1 194	1 172	1 16	1 124	1 150	1 188
280	200	233	1 205	1 202	1 278	1 148	1 184	1 252	1 170	1 172	1 20	1 124	1 150	1 192
349	204	235	1 197	1 202	1 268	1 156	1 184	1 252	1 170	1 172	1 20	1 120	1 150	1 216
309	204	235	1 195	1 202	1 268	1 148	1 186	1 244	1 186	1 172	1 16	1 124	1 142	1 192
277	207	227	1 205	1 200	1 268	1 148	1 184	1 252	1 178	1 174	1 18	1 124	1 146	1 212
278	207	227	1 195	1 200	1 268	1 158	1 186	1 230	1 186	1 168	1 20	1 124	1 150	1 200
459	214	233	1 197	1 200	1 268	1 152	1 184	1 248	1 178	1 174	1 10	1 124	1 142	1 208
460	214	233	1 203	1 216	1 280	1 158	1 184	1 248	1 186	1 184	1 16	1 124	1 146	1 216
270	215	235	1 193	1 200	1 268	1 154	0 188	1 246	1 170	1 160	1 24	1 124	1 150	0 188
259	216	231	1 193	1 200	1 268	0 150	1 184	0 254	1 186	1 172	1 10	1 124	1 150	0 188
272	218	233	1 195	1 204	1 268	1 150	1 186	1 248	1 178	1 172	0 22	1 126	1 146	1 188
273	**218**	**235**	1 193	1 200	1 256	**1 154**	**1 186**	**1 230**	**1 178**	**1 172**	**0 10**	**1 124**	**1 142**	1 188
267	220	233	1 205	1 200	0 268	1 158	1 186	1 244	1 170	1 160	1 14	1 124	1 158	1 188
264	225	227	1 201	1 200	1 268	1 150	1 186	1 242	1 170	0 168	0 10	0 126	1 150	1 192
260	228	229	1 197	1 200	1 268	1 164	1 186	1 250	1 178	1 172	0 10	1 112	1 154	1 188
257	229	227	1 207	1 200	1 256	1 160	1 186	1 246	1 170	1 172	0 14	1 122	1 150	1 208
298	231	233	1 193	1 200	1 280	1 158	1 186	1 232	1 178	1 172	1 14	1 112	1 154	1 188
299	231	229	1 207	1 200	1 268	1 148	1 202	1 220	1 170	1 160	1 12	1 112	1 158	1 208
310	232	233	1 205	1 202	1 268	1 148	1 204	1 220	1 170	1 160	1 14	1 112	1 150	0 188
261	234	233	1 189	1 206	1 272	1 154	1 186	1 250	1 178	1 174	1 24	1 126	1 158	1 188
697	**236**	**235**	**1 197**	**1 200**	**1 268**	**1 154**	**1 186**	**1 230**	1 186	1 174	1 18	1 112	1 150	1 208
698	236	233	1 195	1 202	1 278	1 148	1 184	1 252	1 170	1 172	1 10	1 120	1 150	1 216
456	238	235	1 199	1 216	1 268	1 160	1 184	1 248	1 170	1 172	1 20	1 124	1 150	1 208
457	238	233	1 197	1 200	1 268	1 160	1 186	1 230	1 170	1 172	1 16	1 122	1 150	1 208
312	239	227	1 197	1 202	1 268	1 148	1 184	0 246	1 170	1 178	1 18	1 112	1 150	0 208
342	241	227	1 193	1 202	1 256	1 158	1 184	0 250	1 170	1 174	1 24	1 124	0 146	1 188
347	243	229	1	0 216	1 278	1 150	1 186	1 244	1 170	0 160	0 10	1 112	1 150	1 188
274	243	233	1 193	1 204	1 268	1 160	1 186	1 244	1 170	0 160	0 14	1 124	1 162	1 188
262	246	231	1 193	0 202	0 268	1 148	1 202	1 230	1 170	0 172	1 22	1 124	1 150	1 208
302	247	235	1 195	1 200	1 256	1 150	1 186	1 242	1 170	1 172	1 10	1 126	1 150	1 192
303	247	227	1 195	1 200	1 268	1 158	1 186	1 230	1 178	1 168	1 14	1 128	1 150	1 188
334	248	225	1 183	1 216	1 268	1 152	1 186	1 230	1 178	1 176	1 10	1 126	1 150	0 188
333	248	233	1 205	1 200	1 268	1 152	1 186	**1 230**	**1 178**	**1 172**	**1 10**	**1 124**	1 142	0 188
300	251	227	0 193	1 200	1 278	1 148	1 184	1 252	1 170	1 172	1 18	1 120	1 150	1 216

60015v1/PA

nontransmitted chromosomes

ERSN	KID	sava5	ca211	ca212	1140	59	ca231	ta201	at201	ca225	w3442	ca213	ga201	ga203
301	251	227	0 205	1 200	1 276	1 148	1 184	1 252	1 170	1 172	1 24	1 124	1 150	1 188
285	252	231	1 193	1 200	0 268	1 148	1 184	1 252	1 170	1 174	1 16	1 124	0 150	1 192
258	253	229	1 193	1 200	0 268	1 148	1 186	1 230	1 194	1 172	1 22	1 112	1 154	1 208
467	254	229	1 197	1 216	1 280	1 160	1 184	1 250	1 170	0 172	1 22	1 126	1 154	0 188
266	265	227	0 195	1 202	1 268	1 160	1 186	1 260	1 178	1 174	1 16	1 124	1 158	1 208
485	311	227	1 205	1 200	1 268	1 158	1 184	1 230	1 178	1 184	1 20	1 128	1 154	1 212
313	314	227	1 195	1 202	1 268	1 162	1 186	1	0 170	1 172	1 10	0 124	1 150	1 212
348	314	227	1 195	1 200	1 268	1 148	1 184	1 248	1 170	1 172	1 10	0 128	1 150	1 208
317	316	227	1 201	1 202	1 268	1 152	1 186	1 244	1 170	1 174	1 14	1 112	1 154	1 188
318	319	227	0	0	0 256	0 154	0	0	0	0	0 16	1	0	0
320	321	237	1 201	0 200	0 268	1 154	0 186	0 220	1 170	1 172	0 20	1 124	0 146	0 192
336	326	227	1 193	1 202	1 268	1 154	1 186	1 244	1 170	1 160	1 18	1 124	1 154	1 208
325	326	227	1 201	1 202	1 276	1 148	1 186	1 244	1 170	1 176	1 20	1 126	1 150	1 192
330	329	233	1 197	1 202	1 268	1 148	0 184	1 256	1 178	1 172	1 16	1 124	1 162	1 208
476	331	229	0 199	1 200	0 276	1 154	1	0 244	1 170	0 160	1 10	0 112	0 150	0 188
354	351	233	1 201	0 200	1 268	1 162	1 186	1 248	1 178	1 160	1 22	1 132	1 150	1 188
352	353	225	0 207	1 200	1 268	0 154	1 194	1 220	1 170	1 178	1 18	1 128	1 146	1 192
362	356	231	1 195	1 202	1 268	1 154	1 186	0 230	0 170	0 172	1 10	0 128	1 150	1 208
358	357	235	1 205	1 202	0 256	1 154	0 186	1 230	1 178	1 172	1 10	1 124	0 154	1 216
365	359	233	1 205	1 200	1 268	1 162	1 186	1 248	1 178	1 160	1 22	1 132	1 150	1 188
378	359	231	1 201	1 202	1 268	1 162	1 186	1 230	1 186	1 174	1 18	1 126	1 150	1 188
360	361	227	0 195	1 202	0 268	1 162	1 186	1 250	1 170	1 172	1 18	1 124	1 150	1 212
366	367	227	1 193	1 202	1 268	1 154	1 186	1 230	1 178	1 160	1 10	1 124	1 142	0 188
370	372	227	0 201	1 202	1 268	1 150	1 184	0 244	0 170	1 174	1 14	1 124	0 150	0 188
389	384	231	1 203	1 204	1 272	1 158	1 186	1 244	1 178	1 172	1 18	1 126	1 150	1 200
408	409	229	1 205	1 216	1 276	1 154	1 186	1 244	1 178	1 184	1 28	1 112	1 154	1 196
410	409	229	1 197	1 204	1 272	1 158	1 186	1 244	1 178	1 172	1 18	1 126	1 150	1 188
414	413	227	1 195	1 200	1 268	1 158	1 186	1 244	1 178	0 174	1 18	1 120	1 150	1 188
412	413	235	1 193	1 200	1 256	1 156	1 186	1 246	1 170	1 172	1 10	1 124	1 150	1 212
433	435	227	1 195	1 202	1 268	1 154	1 186	1 242	1 170	1 172	1 10	1 112	1 150	1 204
444	443	235	1 205	1 200	1 268	1 158	1 186	1 232	1 178	1	0 24	1 124	1 158	0 188
551	458	235	1 201	1 206	1 268	1 148	1 184	1 248	1 170	1 174	1 14	1 112	1 158	1 188
472	473	233	1 193	1 200	1 268	1 156	1 186	1 248	1 178	1 184	1 10	1 124	1 146	1 188
482	484	233	0 197	1 200	1 268	1 158	1 182	0 248	1 170	1 174	1 16	1 124	1 150	1 188

nontransmitted chromosomes

ERSN	KID	sava5	ca211	ca212	1140	59	ca231	ta201	at201	ca225	w3442	ca213	ga201	ga203
486	487	227	1 201	1 202	1 256	1 154	1 186	0 230	1 178	1 172	1 10	0 124	1 150	1 188
508	488	233	1 205	1 202	1 268	1 148	1 184	1 220	1 170	1 160	1 24	1 112	1 150	1 188
635	498	227	1 193	1 202	1 268	1 148	1 184	1 254	1 170	1 174	1 16	1 124	1 142	1 188
527	501	229	1 183	1 216	1 280	1 158	1 186	1 230	1 170	1 172	1 22	1 126	1 146	1 212
528	501	225	1 183	1 216	1 268	1 152	1 186	1 242	1 170	1 176	1 10	1 126	1 154	1 208
502	505	235	1 205	1 200	1 268	1 148	1 184	1	0 170	1 174	1 10	1 126	1 150	1 188
517	516		0	0	0	0	0	0	0	0	0	0	0	0
529	531	233	1 205	1 200	0 268	1 158	1 186	1 242	1 170	1 180	1 10	0 128	1 150	1 204
633	536	229	0 201	1 200	1 268	0 154	1 186	1 230	1 178	1 168	0 10	1 124	0 150	1
532	537	227	1 201	1 200	1 268	1 150	1 186	1 242	1 170	0 172	1 10	1 126	1 150	1 192
534	537	235	1 205	1 200	1 268	1 158	1 186	1 232	1 170	0 160	1 24	1 112	1 150	1 208
562	540	229	1 195	1 202	1 268	1 160	1 184	1 250	170	1 160	1 18	1 124	1 150	1 212
539	540	229	1 207	1 200	1 268	1 154	1 194	1 220	1 170	1 178	1 18	1 128	1 158	1 192
576	578	235	1 199	1 200	1 256	1 158	1 186	1 246	1 170	1 174	1 10	1 124	1 158	1 188
579	578	233	1 199	1 200	1 278	1 148	1 186	1 246	1 170	1 184	1 16	1 124	1 150	1 208
582	587	227	1 201	1 202	1 268	1 148	1 202	1 220	1 178	1 184	1 10	1 128	1 150	1 212
580	587	229	1	0 200	1 268	1 154	1 186	1 244	1 170	1 160	1 10	1 126	1 150	1 200
638	637	237	1 203	1 206	1 268	1 154	0 186	1 228	1 170	1 160	1 22	1 126	1 142	0 212
647	649	229	1 195	1 202	1 268	1 154	1 186	1 232	1 178	1 160	1 10	1 124	1 150	1 216
646	649	231	1 201	1 206	1 268	1 154	1 186	1 230	1 178	1 160	1 10	1 124	1 154	1 188
652	653	235	1 201	1 206	1 268	0 154	0 186	1 230	1 178	1 172	1 16	1 126	1 150	1 188
662	661	235	1 209	1 202	1 280	1 154	1 186	1 242	1 178	1 172	1 22	1 126	1 150	1 188
660	661	233	1 183	1 216	1 268	1 158	1 186		0 170	1 160	1 14	1 122	1 150	1 192
666	667	235	1 203	1 202	1 268	1 158	1 186	1 246	1 170	1 174	1 10	1 126	1 150	1 192
668	667	237	1 209	1 202	1 268	1 150	1 186	1 252	1 178	1 172	1 16	1 128	1 150	1 196
670	669	235	1 205	1 200	1 268	1 148	1 184	1 254	1 170	1 174	1 10	1 126	1 154	1 192
671	669	227	1 195	1 200	1 278	1 158	1 186	1 230	1 178	1 168	1 16	1 128	1 154	1 188
678	676	223	1 201	1 200	0 278	1 156	1 200	1 252	1 174	1 174	1 10	1 124	1 150	1 208
730	684	229	1 195	1 200	0 268	1 148	1 198	1 220	1 170	1 174	1 20	1 126	1 150	1 196

ca219	1105	ca209	ca202	1146	166d05	476	
241	1 103	1 173	1 186	1 274	1 316	1 269	1
241	1 85	1 173	1 182	1 270	1 316	1 263	1
243	1 85	1 177	1 192	1 270	0 312	1 265	1
241	1 85	1 173	1 192	1 270	0 312	1 267	1
241	1 85	1 173	1 198	1 274	1 308	1 271	1
245	1 101	1 175	1 196	1 274	1 316	1 267	1
245	1 101	1 177	1 190	1 274	1 312	1 267	1
241	1 85	1 173	1 202	1 270	1 312	1 269	1
235	1 95	1 181	0 190	0 274	1 308	1 267	0
241	1 85	1 173	1 196	1 274	1 304	1 267	0
235	1 103	1 181	1 196	1 274	1 312	1 265	1
235	1 99	1 181	1 196	1 274	1 304	1 271	1
241	1 85	0 173	1 192	1 270	1 312	1 271	1
241	1 85	0 173	0 196	1 270	1 304	1 271	1
235	1 93	1 181	1 196	1 274	1 308	1 269	0
241	1 103	1 177	1 196	1 270	1 316	1 267	1
235	1 97	1 181	1 198	1 274	1 300	1 271	1
245	1 85	1 177	1 192	1 270	1 300	1 271	1
241	1 85	0 173	0 196	1 274	1 308	1 271	1
235	1 95	1 181	1 198	0 274	1 300	1 267	1
241	1 85	1 173	1 196	1 274	1 300	1 267	1
243	1 85	1 177	1 192	1 270	1 312	1 265	1
241	0 99	1 177	1 198	1 270	1 312	1 263	1
241	0 97	1 177	1 196	1 274	1 304	1 275	1
245	1 85	0 177	1 196	1 272	1 308	1 263	0
235	0 99	1 173	0 196	1	0 304	0	0
235	1 101	1 181	1 194	1 274	1 308	1 267	1
241	1 85	1 173	1 196	1 274	1 304	1 271	1
243	1 85	1 177	1 192	1 270	1 312	1 265	0
241	1 85	1 177	1 198	1 270	1 300	0 267	1
241	1 85	1 173	1 196	1 276	1 304	1 271	1
239	1 85	1 181	1 196	1 274	1 300	1 267	0
241	1 99	1 181	1 194	1 274	1 324	1 267	0
241	1 85	1 181	1 196	1 274	1 304	1 267	0
243	1 85	1 177	0 192	1 270	1 312	1 265	1

ca219	1105		ca209		ca202		1146		166d05		476		
235	1	101	1	177	0	200	1	272	1	316	1	267	1
235	0	85	1	173	0	192	1	274	0	308	1	267	1
235	1	101	1	181	1	196	1	274	1	308	1	265	1
245	1	103	1	175	1	198	1	274	1	300	1	271	0
235	1	101	1	181	1	202	1	274	1	316	1	265	1
245	1	85	1	179	1	184	0	270	1	308	1	269	0
241	1	85	1	173	1	192	1	270	1	312	1	269	1
241	1	85	1	173	1	198	1	270	1	308	1	271	1
235	0	101	0	173	0	190	1	274	1	304	0	267	0
245	1	85	1		0		0	274	1	320	1	269	1
	0	103	1	173	1	182	1	274	1	312	1	271	0
241	1	85	1	173	1	182	1	270	1	312	1	273	1
241	1	85	1	177	1	200	1	274	1	308	1	263	1
235	1	85	1	173	1	196	1	270	0	316	1	265	0
241	1	85	1	173	1	182	1	270	1	300	1	265	0
241	1	85	1	173	1	182	1	270	1	308	1	267	1
241	1	87	1	173	1	182	0	272	1	300	1	271	0
245	1	85	0	177	1	198	1	274	1	300	1	271	1
241	0	85	0	173	0	190	0	270	0	312	1	273	0
241	1	85	1	173	1	182	1	270	1	312	1	267	0
241	1	85	1	177	1	192	1	270	1	312	1	267	1
241	1	85	1	173	0	192	1	270	1	308	1	269	0
243	1	85	1	173	0	200	1	274	1	308	0	265	1
243	1	85	1	173	1	190	0	270	0	316	1	273	1
235	0	95	1	173	0	196	1	274	1	312	1	271	1
243	1	85	1	173	0	198	1	270	1	300	1	271	1
241	1	85	1	173	0	196	1	274	1	316	1	267	1
241	1	85	1	173	1	194	1	270	1	316	1	265	1
241	1	85	1	173	1	200	1	274	1	316	1	271	1
241	1	85	1	173	1	194	1	270	1	300	1	271	1
235	1	105	1	181	1	200	1	272	1	316	1	267	1
239	0	101	1	173	1	196	1	274	1	300	1	271	1
241	1	85	1	173	0	192	1	270	0	316	1	265	1
241	1	83	1	173	1	196	1	270	0	304	1	267	0

nontransmitted chromosomes

ca219	1105	ca209	ca202	1146	166d05	476	
241	0 103	1 173	1 192	1 274	1 312	1 267	1
243	1 85	1 173	1 196	1 274	1 308	1 273	1
243	1 85	1 173	1 200	1 274	1 312	1 271	1
241	1 85	1 173	1 182	1 270	0 320	1 267	1
241	1 87	1 173	1 198	1 270	0 312	1 267	1
235	1 97	1 181	1 192	0 274	1 300	1 271	1
	0	0	0	0 272	1		0
235	1 81	1 173	1 182	1 278	1 320	1 261	1
241	1 85	0 173	0 200	1 270	1 304	1 271	1
241	1 85	0 173	1 196	1 270	1 304	1 271	1
235	1 85	0 181	1 194	1 274	1 308	1 267	1
239	1 85	1 173	1 194	1 272	0 316	1 271	1
241	1 85	1 173	1 182	1 272	0 300	1 271	1
241	1 105	1 173	1 192	0 274	1 312	1 267	1
241	1 87	1 173	1 192	0 272	1 304	1 275	1
	0 103	1 173	1 194	1 270	1 316	1 271	1
	0 101	1 173	1 196	1 272	1 308	1 271	1
241	1 87	1 173	1 182	1 274	1 320	1 269	1
241	0 85	1 173	1 194	1 270	1 312	1 267	1
241	0 85	1 173	1 196	1 274	1 300	1 271	1
235	1 99	1 181	1 192	1 274	1 312	1 267	1
235	1 101	1 181	1 196	1 272	1 300	1 271	1
235	1 85	1 179	1 196	1 274	1 312	1 271	1
241	1 85	1 173	1 192	1 270	1 312	1 271	1
241	1 87	1 173	1 182	1 270	1 316	1 273	1
235	1	0 181	1 196	1 274	1 300	1 271	1
239	1 85	0 181	1 196	1 276	1 300	1 267	1
241	1 83	1 177	1 182	1 276	1 308	0 269	1
235	0 93	1 173	0 202	1 272	0 300	1 273	1
			79				

Figure 10

cont	sava5 (miss)	ca211	ca212	1140	59	ca231	ta201	at201	ca225	w3442	ca213	ga201	ga203	
98		193	1 200	1	0 156	1 186	1 230	1 178	1 176	1 10	1 126	1 150	1 208	1
98	17	193	1 216	1	0 148	1 186	1 244	1 178	1 172	1 18	1 124	1 150	1 208	1
99		193	1 206	1 268	1 150	1 184	1 252	1 178	1 172	1 20	1 124	1 142	1 204	1
99		195	1 200	1 268	1 154	1 184	1 220	1 170	1 170	1 10	1 128	1 150	1 188	1
101		189	1 206	1 272	1 154	1 186	1 260	1 178	1 174	1 20	1 126	1 158	1 216	1
101		203	1 200	1 268	1 150	1 186	1 244	1 170	1 160	1 14	1 122	1 150	1 188	1
102		195	1 202	1 268	1 150	1 202	1 220	1 178	1 172	1 24	1 124	1 150	1 212	1
102		205	1 200	1 268	1 162	1 186	1 248	1 178	1 160	1 22	1 132	1 150	1 188	1
104		195	1 200	1 268	1 154	1 186	1 244	1 170	1 160	1 10	1 126	1 150	1 188	1
104		203	1 216	1 268	1 156	1 186	1 244	1 186	1 174	1 14	1 126	1 150	1 192	1
105		193	1 202	1 268	1 156	1 186	1 244	1 170	1 172	1 10	1 126	1 150	1 188	1
105		201	1 216	1 268	1 148	1 186	1 246	1 194	1 172	1 16	1 124	1 150	1 188	1
107			0 206	1 268	1 154	1 186	1 246	1 170	1 176	1 22	0	0 154	1 188	1
107			0 202	1 274	1 150	1 184	1 246	1 170	1 174	1 16	0	0 150	1 216	1
108		201	0 200	1 268	1 162	1 186	1 230	1 178	1 172	1 22	1 126	1 150	1 188	1
108		195	0 202	1 280	1 154	1 186	1 242	1 178	1 172	1 22	1 126	1 150	1 192	1
110		199	1 218	1 268	1 160	1 184	1 248	1 170	1 172	1 16	0 124	1 150	1 208	1
110		205	1 200	1 268	1 148	1 184	1 254	1 170	1 174	1 10	0 126	1 150	1 188	1
111		193	1 202	1 268	1 154	1 186	1 232	1 178	1 160	1	0 124	1 150	1 188	1
111		191	1 202	1 268	1 150	1 184	1 252	1 170	1 160	1	0 128	1 150	1 188	1
114		207	1 202	1 268	1 150	1 200	1 220	1 170	1 174	0 24	1 126	1 150	1 212	1
114		195	1 200	1 278	1 154	1 186	1 252	1 178	1 172	0 18	1 124	1 150	1 192	1
113		191	1 202	1 276	1 150	1 184	1 250	1 170	1 174	0 22	1 124	1 146	1 216	1
113		207	1 216	1 268	1 150	1 186	1 244	1 170	1 172	0 16	1 124	1 150	1 192	1
116		193	1 202	1 268	1 154	1 186	1 230	1 178	1 172	1 10	1 124	0 150	1 188	1
116		195	1 202	1 268	1 154	1 186	1 248	1 170	1 172	1 10	126	0 150	1 212	1
117		201	1 200	1 268	1 154	1 186	1 232	1 178	1 172	1 10	1 124	0 142	1 212	1
117		195	1 202	1 268	1 160	1 186	1 256	1 178	1 174	1 16	126	0 158	1 212	1
119		193	1 200	1 270	1 162	1 186	1 244	1 170	1 172	1 18	0 124	1 150	1 216	1
119		193	1 206	1 268	1 154	1 186	1 230	1 178	1 172	1 10	0 126	1 150	1 188	1
120		193	1 216	1 276	1 158	1 186	1 242	1 178	1 174	1 18	0 112	1 154	1 192	1
120		203	1 204	1 272	1 158	1 186	1 244	1 178	1 172	1 10	0 126	1 150	1 200	1
122		183	1 200	1 268	1 154	1 186	1 242	1 178	1 160	1 16	1 124	1 150	1 204	1
122		195	1 218	1 268	1 156	1 186	1 232	1 178	1 160	1 26	1 124	1 150	1 188	1

controls

controls

cont	sava5	ca211	ca212	1140	59	ca231	ta201	at201	ca225	w3442	ca213	ga201	ga203
123		193	1 200	1 268	1 150	1 184	1 252	1 170	1 160	1 10	1 126	1 154	0 188
123		195	1 216	1 268	1 154	1 184	1 232	1 170	1 160	1 20	1 112	1 150	0 192
125		203	1 200	1 268	1 148	1 184	1 252	1	0 174	1 18	1 124	1	0 212
125		205	1 202	1 268	1 148	1 188	1 250	1	0 172	1 16	1 124	1	0 192
126		205	1 200	1 268	1 148	1 186	1 248	1 170	1 160	1 14	1 128	0 150	1 188
126		195	1 204	1 268	1 150	1 186	1 246	1 178	1 172	1 22	1 126	0 150	1 208
128		193	1 200	1 256	1 158	1 186	1	0 170	1 174	1 14	1 112	1 158	1 188
128		191	1 200	1 268	1 160	1 184	1	0 170	1 172	1 18	1 122	1 150	1 208
129		193	1 206	1 256	1 154	1 186	1 244	1 170	1 174	1 10	1 112	1 158	1 188
129		195	1 216	1 268	1 150	1 184	1 250	1 170	1 172	1 14	1 126	1 150	1 192
131		201	0 200	1 268	1 154	1 186	0 252	0 186	0 176	0 18	1 126	1 150	1 188
131		197	0 200	1 268	1 150	1 184	0 244	0 170	0 172	0 10	1 126	1 150	1 188
132		205	0 200	1 268	1 148	1 186	0 252	0 186	0 174	0 18	1 124	1 150	1 212
132		203	0 200	1 268	1 158	1 184	0 248	0 170	0 172	0 18	1 124	1 158	1 208
134		193	1 216	1 268	1 148	1 186	1 220	1 170	1 174	1 14	1 124	1 150	1 208
134		205	1 202	1 266	1 160	1 186	1 230	1 194	1 172	1 22	1 112	1 154	1 208
135		193	1 202	1 268	1 154	1 186	1 244	1 170	1 160	1 18	1 124	1 154	1 208
135		205	1 202	1 268	1 154	1 184	1 230	1 178	1 184	1 20	1 128	1 154	1 208
138		193	1 202	1 268	1 154	1 186	1 230	1 178	1 172	0 10	1 124	1	0 216
138		207	1 200	1 280	1 148	1 184	1 252	1 178	1 174	0 20	1 126		0 216
137		193	1 206	1 268	1 154	1 186	1 230	1 178	1 172	0 10	1 126	1 150	1 192
137		201	1 216	1 270	1 148	1 184	1 256	1 186	1 174	0 10	1 126	1 150	1 212
144			0 200	1 256	1 154	1 186	1	0	0 174	1 10	1 126	1 150	1 208
144			0 206	1 268	1 154	1 186	1	0	0 176	1 22	1 124	1 150	1 188
68		195	1 202	1 268	1 164	1 186	1	0	0 172	1 22	1 126	1 150	1 208
68		193	1 202	1 268	1 160	1 186	1	0	0 172	1 18	1 122	1 150	1 208
69		195	1 218	1 268	1 148	1 186	1 246	1	0 160	1 10	1 124	1 146	1 208
69		201	1 216	1 268	1 158	1 186	1 230	1	0 172	1 20	1 124	1 150	1 204
72		193	1 200	1 268	1 148	1 184	1	0 170	1 174	1 16	1 124	1 150	1 188
72		193	1 206	1 256	1 156	1 186	1	0 170	1 172	1 10	1 124	1 150	1 192
71		193	1 216	1 268	1 146	1 192	1 248	1 170	1 174	1 16	1 124	1 154	1 196
71		193	1 206	1 256	1 156	1 186	1 232	1 170	1 174	1 10	1 126	1 150	1 212
74		195	1 218	1 268	1 148	1 186	1 246	1 170	1 160	1 10	1 124	1 154	1 216
74		205	1 200	1 268	1 158	1 186	1 222	1 170	1 160	1 24	1 112	1 154	1 188

cont	sava5	ca211	ca212	1140	59	ca231	ta201	at201	ca225	w3442	ca213	ga201	ga203	
75		217	1 216	1 264	1 150	1 186	1 250	1 170	1 180	1 12	1 124	1 150	1 192	1
75		205	1 204	1 268	1 154	1 186	1 244	1 170	1 172	1 16	1 124	1 146	1 192	1
78		201	1 216	1 268	1 148	1 186		0 174	1 172	1	0 124	1 150	1 192	0
78		201	1 202	1 268	1 162	1 186		0 170	1 174	1	0 126	1 150	1 188	0
77		201	1 206	1 268	1 158	1 184	1 246	1 170	1 160	1 22	1 124	1 150	1 192	0
77		195	1 202	1 268	1 152	1 186	1 232	1 178	1 174	1 20	1 122	1 146	1 188	0
80		193	0 202	0 268		0 186	1 250	1 178	1 160	1 10	1 124	1 150	1 208	0
80		195	0 200	0 268	1	0 186	1 244	1 178	1 172	1 28	1 124	1 150	1 208	1
81		193	1 202	0 268	1 156	1 186	1 246	1 194	1 172	1 10	1 126	1 150	1 188	1
81		193	1 200	0 268	1 148	1 184	1 258	1 186	1 174	1 10	1 124	1 150	1 208	1
84		193	1 202	1 268	1 154	1 186	1 246	1 170	1 172	1 14	1 126	1 158	1 188	1
84		207	1 202	1 268	1 164	1 186	1 244	1 170	1 178	1 10	1 124	1 150	1 188	1
83		209	1 200	1 270	1 148	1 184	1 230	1 178	1 172	1 26	1 124	1 150	1 208	1
83		207	1 200	1 268	1 158	1 186	1 248	1 170	1 174	1 10	1 112	1 146	1 192	1
86		195	1 202	1 268	1 158	1 186	1 244	1 170	1 160	1 14	1 124	1 158	1 208	1
86		205	1 202	1 278	1 148	1 184	1 260	1 170	1 172	1 20	1 120	1 150	1 188	1
87		197	1 200	1 268	1 158	1 186	1 230	1 178	1 172	1 10	1 124	1 158	1 188	1
87		193	1 200	1 268	1 154	1 190	1 242	1 170	1 172	1 16	1 126	1 154	1 188	1
90		205	1 200	1 268	1 158	1 186	1 250	1 170	1 172	1 18	1 124	1 158	1 208	1
90		193	1 200	1 268	1 154	1 186	1 246	1 186	1 172	1 10	1 124	1 150	1 188	1
89		207	1 202	1 270	1 168	1 186	1 232	1 178	0 176	1 22	1 126	1 154	1 212	1
89		193	1 202	1 268	1 154	1 190	1 252	1 170	0 172	1 16	1 126	1 150	1 188	1
92		193	1 200	1 268	1 148	1 184	0 244	1 170	0 174	1 10	1 124	1 150	1 208	1
92		193	1 202	1 256	1 154	1 186	0 230	1 178	0 172	1 10	1 124	1 154	1 188	1
93		203	1 216	1 268	1 156	0 186	0 248	1 170	1 174	1 14	1 126	1 150	1 204	1
93		205	1 200	1 268	1 148	0 184	0 230	1 178	1 174	1 10	1 126	1 150	1 188	1
95		197	1 216	1 268	1 158	0 186	1 252	1 178	1 174	1 20	1 126	1 150	1 192	0
95		205	1 202	1 268	1 150	0 184	1 230	1 178	1 160	1 10	1 126	1 150	1 188	0
96		209	1 200	1 278	1 162	0 186	1 256	1 170	1 160	1 14	1 128	1 150	1 192	0
96		205	1 200	1 268	1 148	0 186	1 230	1 178	1 160	1 14	1 128	1 150	1 188	0
140			0	0 270	1	0	0 244	1	0	0 10	1	0 150	1 188	1
140			0	0 278	1	0	0 254	1	1	0 10	1	0 158	1 188	1
141		201	0 200	1 272	1	0	0 244	1 170	1 172	1 10	1	0	0 216	1
141		193	0 200	1 270	1	0	0 254	1 170	1 160	1 10	1	0	0 212	1

controls

cont	sava5	ca211	ca212	1140	59	ca231	ta201	at201	ca225	w3442	ca213	ga201	ga203	
143		1 93	1 200	1 278	1 148	1 184	1 252	1 170	1 184	1 18	1 124	1 150	0 192	1
143		1 95	1 200	1 268	1 158	1 186	1 248	1	178	1 18	1 124	1 146	0 188	1

ca219	1105	18SCA20	SC	KID
241	1 missing	173	1	100
241	1	177	1	100
235	1	173	1	100
245	1	175	1	100
235	1	173	1	103
235	1	181	1	103
241	1	173	1	103
241	1	173	1	103
235	1	181	1	106
241	1	173	1	106
241	1	173	1	106
241	1	173	1	106
241	1	173	1	109
241	1	173	1	109
241	1	173	1	109
245	1	175	1	109
235	1		0	112
235	1		0	112
243	1	181	0	112
235	1	173	0	112
241	1	173	1	115
241	1	173	1	115
241	1	173	1	115
241	1	173	1	115
241	1	173	1	118
241	1	173	1	118
241	1	173	1	118
241	1	177	1	118
241	1	173	1	121
241	1	173	1	121
241	1	173	1	121
235	1	181	1	121
	0	173	1	124
	0	173	1	124

ca219	1105	18SCA20	SC	KID
241	1	173	1	124
241	1	177	1	124
241	1	173	1	127
241	1	173	1	127
243	1	173	1	127
239	1	173	1	127
235	1	181	1	130
235	1	181	1	130
241	1	173	1	130
241	1	173	1	130
243	0	181	0	133
235	0	173	0	133
245	0	181	0	133
235	0	173	0	133
243	1	173	1	136
235	1	181	1	136
241	1	173	1	136
241	1	173	1	136
241	1		0	139
243	1		0	139
235	1	181	0	139
241	1	177	0	139
241	1	173	1	145
241	1	173	1	145
241	1	173	1	70
245	1	177	1	70
241	1	173	1	70
243	1	175	1	70
243	1	173	1	73
241	1	173	1	73
235	1	181	1	73
241	1	173	1	73
241	1	173	1	76
235	1	181	1	76

ca219	1105	18SCA20	SC	KID
241	1	175	1	76
235	1	177	1	76
241	1	173	1	79
241	1	177	1	79
241	1	173	1	79
241	1	181	1	79
241	1		0	82
241	1		0	82
241	1	173	1	82
241	1	173	1	82
241	1	173	1	85
241	1	173	1	85
241	1	173	1	85
241	1	173	1	85
	0	173	1	88
	0	173	1	88
235	1	173	1	88
235	1	181	1	88
245	1	173	1	91
241	1	173	1	91
241	1	181	1	91
241	1	173	1	91
241	1	173	1	94
241	1	173	1	94
241	1	177	1	94
235	1	181	1	94
241	1	173	1	97
245	1	173	1	97
221	1	173	1	97
241	1	173	1	97
	0		0	142
	0		0	142
241	1	173	1	142
241	1	173	1	142

ca219	1105	18SCA20	SC	KID
241	1	177	1	145
235	1	181	1	145

AHR RESULTS IN DISEASE CHROMOSOMES

	SAVA5	CA211	CA212	18S1140	18S59	TA201	CA231	AT201	CA225	W344
SAVA5	■	0.04 2%			0.07 4%					
CA211	2-3	■								
CA212			■							
18S1140				■	0.70 12%		1.92 30%		1.28 20%	
18S59	2-6				■	2.11 10%	2.45 18%	0.53 10%	1.16 12%	4.18 17%
TA201				2-4	4-3	■	0.68 3%	1.34 10%	0.02 2%	0.68 8%
CA231				2-2	4-2	2-4	■		0.89 16%	
AT201					4-2	3-2	2-3	■	0.18 4%	
CA225				2-3	4-3	3-3	2-3	1-1	■	0.03 4%
W3442					4-1	3-1	2-1	2-1		■

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHODS FOR TREATING BIPOLAR MOOD DISORDER ASSOCIATED WITH MARKERS ON CHROMOSOME 18P

the specification of which:

[] is attached hereto.

[X] was filed on November 24, 1997, and identified as Attorney Docket No. UCAL-250/02US.

[] was filed on _____, as Application Serial No.

and

[] the amendment(s) of which were filed on .

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under title 35, United States Code, Section 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s) (Country) (Number) (Day/Month/Year Filed)   Priority Claimed (Yes/No)

21419491

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

_____60/023,438_____   _____August 23, 1996_____
(Application Number)   (Filing Date)

_____   _____
(Application Number)   (Filing Date)

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Section 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Appl. Ser. No.	Filing Date	Status (Pat'd./Pend./Aband.)
08/916,683	8/22/97	Pending

I hereby appoint:

Richard L. Neeley	30,092	Marcella Lillis	36,583
Willis E. Higgins	23,025	Craig P. Opperman	37,078
Tom M. Moran	26,314	Melya J. Hughes	38,696
John W. Girvin, Jr.	22,706	Brian Lewis	32,502
Nina M. Ashton	37,273	Gurjeev K. Sachdeva	37,434
Jackie N. Nakamura	35,966	Alexandra J. Baran	39,101
Peter R. Leal	24,226	Saul A. Seinberg	24,840
James A. Bradburne	38,389	David R. Stevens	38,626

my attorneys and agents with full power of substitution and revocation to prosecute my above-identified application for Letters Patent and to transact all business in the Patent Office connected therewith.

I further direct that correspondence concerning this application be directed to

COOLEY GODWARD LLP
Five Palo Alto Square
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Palo Alto, California 94306-2155
Attention: Patent Group
Telephone (650) 843-5000.

21419491

2.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Full name of third inventor: Pedro Leon

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Citizen of: United States of America

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Full name of sixth inventor: Lynne Allison McInnes

Inventor's signature _____   Date _____

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Citizen of: Canada

Post Office Address: Same as above

Full name of seventh inventor: Susan K. Service

Inventor's signature _____   Date _____

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4.